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BIOLOGICAL BULLETIN

ON THE PRESENT STATUS OF THE CHONDRIOSOME-PROBLEM.[1]

J. DUESBERG.

Before I come to the subject of this address, I want to make a short incursion into the domain of nomenclature. As I recently pointed out, the word "mitochondrium" designates a body of granular form. Since those who use it as a general denomination have in mind certain bodies which very often appear as filaments, they are led to speak of "filamentous mitochondria," something which is inconceivable. Besides, using a term in a new sense adds greatly to the confusion already existing in the nomenclature, much more than the use of a new term. In 1915 I reverted to the use of the word "chondriosome," for although there may be some objection to it, in the present state of things it has two distinct advantages—one, that it does not preclude anything as to the form of the bodies which it is supposed to designate; the other, that it is already extensively used, so that its further adoption would bring us very near to that much-to-be-desired unification in our nomenclature.

Speaking of the present status of the chondriosome-problem, I have to speak of what has been done in this particular field of cytological research, and also of what is still to be done. There is much to say on either topic, since the number of observations published on chondriosomes is enormous and since an agreement on some the most fundamental questions is as yet far from being reached. Owing to lack of time, however, my account must necessarily be confined to a few essential points.

Beginning with what has been accomplished, one of the most gratifying results for the cytologist who works in that field is the

[1] Lecture delivered at Woods Hole Marine Biological Laboratory, Mass., July 23, 1918.

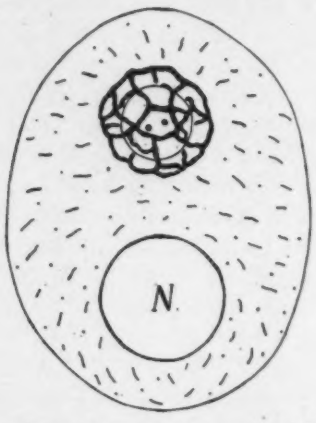
following. After the publication of Benda's somewhat complicated method, it was a common opinion that what that method brings into evidence is only a distorted appearance of the cellular structures. Thus, however, were disregarded a number of observations, some of which belong to the earliest period of cytological investigation: I shall limit myself to mention von Brunn's observations on the formation of the spiral filament in the spermatozoön of the mouse, von la Valette St. George's work with dahlia-violet applied in vivo, Michaelis's discovery of the peculiar properties of the janus-green.[1] Quite lately the chondriosomes have been extensively studied in the living cell, especially in tissue cultures: prominent investigators in this field are W. H. and M. R. Lewis. These recent observations have confirmed what the old ones already taught—that chondriosomes are not artefacts and that they appear in the fixed and stained preparations very much the same as they are in the living cell.

Another important result of the recent studies on chondriosomes is a simplification in our conception of the morphology of the protoplasm. When Benda first described his "mitochondria" (at that time, he was convinced that the granular form was the rule), he thought he had discovered a new cellular organule. The question soon arose: what is the relationship between these organules and other constituents of the cell, such as Flemming's fila, Maggi's plastidules, Altmann's bioblasts, Boveri's archoplasmic granules, etc.? Is there any relation between these bodies or are they entirely different? In the last supposition, the structure of the cytoplasm appeared as something so complicated that many, especially under the influence of Fischer's experiments, cleared the field by designating as an artefact any structure which appeared after fixation. We know now, principally as the result of Meves's exertions, that all these elements are one and the same thing. Flemming's filaments (at least those which he described in the resting cell, in contradistinction to the mitome of the dividing cell), Maggi's plastidules found by that author and by the brothers Zoja, his pupils, in practically

[1] Probably the reason why janus-green has until lately been so little in favor among cytologists is that a number of dyes delivered under that name are either some other product, or a substance mixed with impurities, which may be toxic.

all tissues, Altmann's bioblasts whose discovery unfortunately led their author to theoretical considerations which threw disrepute on his observations, Boveri's archoplasmic granules which in the dividing egg of *Ascaris megalocephala* surround the centrosphere,—all these things are identical with Benda's mitochondria and with our chondriosomes. We know further that the morphology of that same substance in the different cells, or even in a given cell at different periods of its life, is subject to great variations. Thus the controversy between the partisans of an exclusively filamentous structure of the protoplasm and those who believed only in granules, has come to an end. The morphological variations of chondriosomes in one cycle of life are especially conspicuous in the seminal cells of many invertebrates. There, in many species, the chondriosomes of the spermatogonia are granules; in the spermatocytes, the granules flow together and build up filaments and finally in the spermatid, a further coalescence very often takes place, the result of which is the formation of a single, compact, bulky body, well-known as von la Valette St. George's "Nebenkern." A reverse process is also observed. To return to an example already given, Boveri's archoplasmic granules in the dividing egg of *Ascaris* are derived from bodies which have, in the young ovocyte, a filamentous form. One should be warned however against reuniting all protoplasmic structures in one single group, as some have done. As far as we know, there is no relationship between the chondriosomes and the idiosome, nor between the chondriosomes and most of the elements designated as Golgi's apparatus. It is becoming more and more clear in my opinion that the latter denomination should be reserved to that element which in most cells, especially in young cells and during the period of rest, is closely connected with the idiosome and which has been described under various names, such as Centrophormien, Zentralkapseln, formazioni periidiozomici, idioectosome, etc. If I had to express in a schema the structure of a young resting cell, I would propose this one, (s. next p.) which in my opinion, corresponds to the present state of our knowledge. It should be added here that, for the majority of investigators, including myself, the chondriosomes belong to the cytoplasm. Quite recently, the question of their

nuclear origin has been brought up again by Schreiner. Taking for granted that Schreiner's description be accurate, nothing proves however that his interpretation be correct, for the process described as an expulsion of substance from the nucleus might just as well be the reverse. And even if we accept Schreiner's interpretation, we find that the question of the origin of the



N, nucleus; above, idiosome with centrioles, surrounded by the apparatus of Golgi. In the protoplasm, chondriosomes.

chondriosomes has really not been touched, since chondriosomes are present in all cells before the process which Schreiner describes takes place.

Finally, the tremendous amount of work published in recent years has revealed the presence of chondriosomes in practically all cells, animal and vegetal. The only animal cells that constitute an exception are the superficial cells of the epiderm: that is, cells whose protoplasm is entirely differentiated and transformed into a new substance. Whether chondriosomes exist in the adult red blood-corpuscle might perhaps be questioned also. It is not impossible that the presence of hæmoglobin, which is precipitated by the fixing reagents, be responsible for the negative results obtained by most authors. But, one may well ask, how can the cytologist decide whether he is in presence of chondriosomes or not? A number of criteria may be applied

in this connection: the behavior towards preserving reagents and dyes; or better still, the evolution of a given cell-constituent, as for instance in the seminal cells; or the continuity of a given structure from the embryonic cell to the adult stage. If however our objector means to say that it may be difficult in a given case to decide whether or not a certain structure is a chondriosome, then I heartily agree with him, but I fail to see therein a reason for general scepticism. Rather it should prove a stimulus for further investigation. It should not be forgotten that the chondriosomes are by no means the only structures that may be hard to identify in certain instances. Indeed, sometimes we have great difficulty in recognizing a nucleus. Take for example the controversy on the so-called Blochman's nuclei in the egg of certain insects, or (as a personal illustration) the divergence of opinion between Retzius and myself as to what part of the spermatozoön of *Ciona* is the nucleus. In this very field (spermiogenesis) the same difficulty is met with constantly when we attempt to study atypical forms.

I come now to the second part of this address: what is still to be done in the investigation of the chondriosomes? Since brevity is essential, I shall limit myself to the consideration of two questions, both of great importance *i. e.*, the rôle played by the chondriosomes in the process of differentiation and their rôle in fertilization. Meves was the first to express the opinion that the chondriosomes of the embryonic cell represent an indifferent material, susceptible of various differentiations in the different tissues. Since that time a number of papers supporting this idea have been published, of which I shall only mention those on the development of the collagenous fibrils (Meves), of neurofibrils (Hoven) and of myofibrils (Duesberg). The same idea is also and very strongly supported by a number of observations concerning the processes of differentiation in vegetal cells. My own observations on myofibrillogenesis have found confirmation in different quarters and so far no specific criticism has been expressed. On the other hand, the conclusions concerning the chondriosomal origin of the neurofibrils and of the collagenous fibrils have been criticized, respectively by Cowdry and M. R. Lewis. I frankly admit that Cowdry's arguments are worth

being very carefully considered, but I should perhaps be allowed at the same time to venture the question whether there is really such a problem as the origin of neurofibrils; in other words, whether the neurofibrils really exist in the living cell. As to the fibrils of the connective tissue, a detailed criticism of M. R. Lewis's paper is out of place here, but I want however to point out that little effort has been made by that author to demonstrate that the fibrils appearing in her tissue-cultures are collagenous fibrils. Yet, I gather from Baitsell's observations that great care should be taken in interpreting such fibrillar structures. Personnally, I have not found in my recent investigations on development, normal and regenerative, any reason for changing my mind. I can clearly see however, how interesting it would be to apply the experimental method to the solution of these problems: experiments on regeneration of tissues, on centrifuged eggs, on the behavior of cells under abnormal and pathological conditions and under the influence of poisonous substances, etc., a field which so far has been explored very little.

The second question I want to consider is this: are the chondriosomes an idioplasmic substance? This opinion was first expressed by Benda. It found strong support in Meves's discovery of chondriosomes in all embryonic cells and later in that author's description of the behavior of the male chondriosomes in *Ascaris* during fertilization. The question is so important that it should be considered with greatest care and at some length.

At the basis of the hypothesis of the idioplasmic nature of the chondriosomes is the idea in which the protoplasm plays a rôle in the transmission of hereditary characteristics. This opinion has always been that of a number of biologists, although a minority, and it has been supported by experimental researches, namely by the well-known experiments made by Godlewsky. The question then arises: what part of the spermatozoön represents the protoplasmic idioplasm? It cannot be the axile filament, which is not constant and is obviously an organ of motility; it cannot be the headcap, nor the acrosome, since these are also inconstant, in some cases (marsupials) only transitory parts of the spermatozoön; nor do I see that any argument can be brought forth in favor of the idioplasmic nature of the centrioles, or of

the small amount of protoplasmic ground-substance which, in the mammalian spermatozoön, forms a thin covering over the head and the proximal part of the tail. But there is another constituent which the recent studies have shown to be constant in all spermatozoa of all species, from the lowest to the highest groups. These are the chondriosomes. The same studies have shown also that the chondriosomes can assume in the ripe spermatozoön a shape and location which vary greatly in the different species. One used to say that the chondriosomes form a sheath around the "middle-piece" of the spermatozoön. This is a very loose, and in many cases an entirely incorrect statement. Loose, since under the term "middle-piece" we designate parts that have nothing in common: there is no homology whatsoever between the so-called "middle-piece" of the spermatozoön of an echinoderm, of a selachian (or an amphibian) and of a mammal. In many cases it is also an incorrect statement. In a number of higher vertebrates, especially in mammals, the chondriosomes actually form a sheath around what is customarily called the "middle-piece" of the spermatozoön; at the same time, the shape of that sheath varies a good deal, from isolated granules to discs or a spiral filament. In lower vertebrates (in many fishes) and in a great number of invertebrates (many molluscs, certain worms, etc.), the chondriosomes are represented by a small number of granules located on the posterior part of the head and surrounding the origin of the tail. A third type often met with, especially in insects, is represented by a long, thin sheath, which envelops the axile filament; this sheath is derived from the so-called "Nebenkern," which usually breaks in two, the pieces afterwards becoming elongated as the tail grows. But besides these three main types there exists also an infinity of other forms. Quoting at random I would mention the spermatozoa of *Ascaris*, *Phallusia*, *Ciona*, of many worms, crustacea, molluscs, etc. It is consequently impossible, in the presence of the ripe spermatozoön only, to decide what part of it is formed by the chondriosomes[1] and only a careful study of spermiogenesis can solve the problem. Indeed such a study should be undertaken

[1] This is a reason why I cannot, without further investigation, consider F. R. Lillie's observations on fertilization in *Nereis* as demonstrating that in that species the male chondriosomes do not penetrate into the egg.

every time one has to deal with atypical forms of spermatozoa as a preliminary to the study of fertilization.

The chondriosomes being a constant constituent of the spermatozoön, have we any clue as to their significance? Opinions differ on this point. Benda is responsible for a hypothesis according to which the chondriosomes are in some way connected with the property of motility. This hypothesis reappears occasionally, and in my opinion very unfortunately, since it is not supported by anything and is contradicted by a number of facts. Of course, one can well imagine how such an idea might be suggested by the appearance of the spiral filament of certain spermatozoa, just as a rather naïve theory of muscular contraction was once built upon the conception that the myofibrils were shaped like a spring. But I fail utterly to see how other forms of the chondriosomal sheath, such as the small granules existing in so many lower vertebrates and in many invertebrates, could suggest in any way a relation with the motility of the spermatozoön. Furthermore, Benda's hypothesis is in contradiction to Meves's experiments of merotomy on spermatozoa and to the presence of chondriosomes in those spermatozoa that make no active movements, such as the spermatozoa of decapods. The same facts disagree also with the idea expressed by Regaud, for whom "le chondriome du spermatozoide est moins un matériel héréditaire qu'une partie de la cellule jouant un rôle actuel de fixation et de concentration des substances ambiantes destinées à être consommées lors de la contraction du filament axile."

Another opinion was expressed by Koltzoff. This author considers the chondriosomes as playing the rôle "einer formbildenden Substanz." While this is apparently true for certain spermatozoa he studied, for decapods for instance, I would deny that Koltzoff's interpretation has any general value. On physical grounds it would seem impossible that bodies which are entirely enclosed in a cell, without coming in contact with its surface, could have anything to do with the form of that cell. To return to the type of spermatozoön so common among lower vertebrates and invertebrates, is it possible to imagine that these granules have anything to do with determining the form of the spermatozoön? The answer can only be a negative one.

Finally we come to the hypothesis according to which the chondriosomes represent the idioplasm contained in the protoplasm of the seminal cells. We must find out first what becomes of the male chondriosomes in the process of fertilization. Although one of the first studies of this process, that made on *Ascaris megalocephala* by van Beneden, showed that the whole spermatozoön may enter the egg, the great majority of biologists have accepted the dogma of the monopoly of the nucleus as carrier of the idioplasm. The reasons for that attitude are obvious. First, in most cases, only the nucleus of the spermatozoön was detected in the egg; further, investigators were quite naturally hypnotized by the beautiful karyokinetic figures which they saw formed in the fertilized egg at the expense of the pronuclei. Yet, after van Beneden, a number of other authors (Kostanecki, Nekrassof, Lams, etc., in invertebrates; Fick, Nicolas, Michaelis, Van der Stricht, etc., in vertebrates) have demonstrated that other parts of the spermatozoön are also carried into the egg. The hypothesis in which the chondriosomes represent an idioplasmic substance has given a renewed interest to these observations which had been considered as exceptional cases, and recent observations, made under the impulse of the same hypothesis, are likely to lead us to a reconsideration of our conception of the morphology of fertilization. I need only mention in this connection the case of the sea-urchin. It was generally accepted that only the nucleus and the so-called "middle-piece" penetrate into the egg and that the tail is left without. Recent observations, however, made with special care and a more refined technique, have shown that the tail also enters. Let us not forget, however, that the crucial point to determine is not whether the tail, but whether the chondriosomes are carried into the egg. As I pointed out when speaking of the structure of the spermatozoön, these questions have no connection. One can readily conceive of a type of spermatozoön in which only the head would penetrate, and yet the chondriosomes would enter the egg at the same time. Such a type exists, as a matter of fact, in a number of invertebrates, for instance the ascidians *Phallusia* and *Ciona*.[1]

[1] In these two species, however, the tail also is found in the egg.

We have observations, consequently, showing that the tail of the spermatozoön, or part of it, penetrates into the egg. We come now to the cases where the chondriosomes have actually been found in the egg. Most of these cases have been described recently under the impulse of the chondriosomal theory, and, considering the short time intervening since the publication of that theory, their number is already quite impressive. The actual penetration of chondriosomes into the egg was seen long ago, however, by L. and R. Zoja in *Ascaris*, and later by Vander Stricht in the bat. To these observations have been added, during the last seven years, a number of others: on the sea-urchin, *Phallusia*, *Mytilus* and *Filaria* (Meves) and on *Ciona* (Duesberg). Thus we already have observations on a number of invertebrates (worms, ascidians, echinoderms and molluscs) and vertebrates (mammals and amphibians—for there is no doubt from the observations of Fick and Michaelis that in *Triton* and *Axolotl* also the male chondriosomes are carried into the egg).

While things would thus appear to be in perfect agreement with the theory according to which the chondriosomes are idioplasm, a difficulty, and a very serious one indeed, has arisen. First, Vander Stricht and his pupils found in mammals that the chondriosomal part of the spermatozoön (in the bat, the spiral filament) passes unchanged into one of the first two blastomeres. Similarly in the sea-urchin, the chondriosomal middle-piece is found in one of the daughter-cells after the first division (Meves). So far the difficulty could be met, for there is some reason to believe that the two first blastomeres of the mammalian egg are not equivalent, one of them possibly forming the trophoblast only, the other the embryo, and a similar hypothesis could be formulated concerning the sea-urchin, since the adult is formed of parts only of the original embryo. Meves supposed that the male chondriosomal substance would go over into these cells which build up the definite animal. His further investigations on the fate of the middle-piece, up to 32 blastomeres, show that its fate is variable and that certainly not all cells of the young sea-urchin will receive male chondriosomal substance.

Here lies the real difficulty, and its weight seems overwhelming. I would however warn against too hasty conclusions. A number

of considerations: the constancy of the chondriosomes in the spermatozoön, the lack of a satisfactory hypothesis as to their significance[1] and the fact that in a number of adequately investigated cases they have been found to penetrate into the egg, render, in my opinion, further research necessary before a decision can be reached. The study of fertilization is indeed a difficult one, especially the investigation of such minute constituents of the spermatozoön amid the huge mass of the egg, but it is of such fundamental importance that the investigator will find in it his reward.

[1] Since the middle piece of the spermatozoön does not change its shape nor its volume in the egg of the sea-urchin, it can apparently not be considered as having the value of a chemical factor, such as postulated by Loeb in his theory of fertilization.

THE EFFECT OF SOME FOOD HORMONES AND GLANDULAR PRODUCTS ON THE RATE OF GROWTH OF PARAMECIUM CAUDATUM.[1]

MARY H. CHAMBERS.

The animal body is adjusted to live upon plant or animal tissue. It is generally accepted now that the adequate diet must contain more than protein, carbohydrate, and fat, namely, an accessory factor or vitamin. Recent investigations upon dietaries of infants have shown the importance of some of the accessory factors. Hopkins (1912) added yeast to a diet of purified food stuffs and found that it accelerated the growth of rats. The same result was obtained later by Funk and Macallum (1915). More recently Osborne and Mendel (1917) have shown that the addition of yeast to the ration not only causes an increase in rate of growth of rats, but it acts as a stimulus to the appetite, and when removed from the diet growth ceases and decline ultimately follows. As yeast supplies a definite substance for growth so also potato juice acts as a corrective in cases of malnutrition. With these results in mind a series of experiments was undertaken to determine whether or not extracts of yeast and potato juice added to the diet of *Paramecium* produced any marked effect on the rate of division which according to Calkins (1902) is the index to vitality. The investigations were carried on under the direction of Dr. Florence Peebles, to whom the writer is indebted for suggestions and assistance.

A first set of experiments was made upon a pure line of *Paramecia* which had been maintained for several months on a diet of malted milk.

The solutions of yeast and potato which were employed were 1 per cent., and were made with distilled water. There were two methods of preparation with each. According to the first method the compressed yeast cake was merely dissolved in water, in the second, the yeast was first ground in order to allow

[1] From the Biological Laboratory, Bryn Mawr College.

the contents of the cells to enter the solution. One potato solution was boiled and strained, the other was strained raw. The controls were maintained in .2 per cent. solution malted milk. It was customary to feed the control and experimental lines an equal quantity of freshly made solutions every other day. The same food pipette was always used. Isolating pipettes were sterilized in boiling water each time used to avoid contamination in change of food and to kill any *Paramecia* that might have remained in the pipette. Such sterilization is not sufficient to destroy bacteria. All *Paramecia* were kept in depression slides in a moist chamber at constant temperature.

I. Experiments with Food Hormones.

The following tables show the number of divisions in several control and experimental lines. This experiment was carried on for twelve days and a record kept of each daily division. The table shows the data averaged in periods of six days each. Varying proportions of food substances were used. Although the total quantity of food was always the same in both lines, one set of experiments contained two drops of raw potato solution and one drop of the basic food substance—malted milk—with a control of three drops of malted milk;—and another—three drops of raw potato solution with a control of four drops malted milk.

Table I.

Potato (Raw). Daily Division Average For Six Day Periods.

Period.	Control.	Potato, Two Drops.	Control.	Potato, Three Drops.
1	5.5	7.5	4	7
2	8	6	5.5	7.5
Total	13.5	13.5	9.5	14.5
Average	1.11	1.11	.78	1.2

With raw potato the average rate of growth in the weaker solution was practically the same as that of the control while the rate of division for the stronger extract exceeded that of the control.

TABLE II.

POTATO (BOILED). Daily Division Average For Six Day Periods.

Period.	Control.	Potato, Two Drops.	Control.	Potato, Three Drops.
1	6.5	4	7	7
2	6	8.5	6	5.5
Total	12.5	12.5	13	12.5
Average	1.03	1.03	1.08	1.03

It will be observed that when the potato extract was boiled the average rate of division was about the same for the weaker and stronger solutions.

A similar experiment was carried on at the same time with yeast. Two and three drops each of the whole and ground yeast were used.

TABLE III.

YEAST (WHOLE). Daily Division Average For Six Day Periods.

Period.	Control.	Yeast, Two Drops.	Control.	Yeast, Three Drops.
1	7	6	4.5	7
2	4	7	3.5	8.5
Total	11	13	8	15.5
Average	.91	1.08	.66	1.28

As this table shows, the individuals in the solution containing whole yeast divided more rapidly than the control.

TABLE IV.

YEAST (GROUND). Daily Division Average For Six Day Periods.

Period.	Control.	Yeast, Two Drops.	Control.	Yeast, Three Drops.
1	3	7.5	6	5
2	0	9	4.5	8.5
Total	3	16.5	10.5	13.5
Average	1.5	1.37	0.86	1.11

In this case a decided increase in the rate of division is shown when ground yeast is added to the diet.

A second set of experiments was made upon four races of *Paramecia* as follows: Race A had been in spring water in the

laboratory for several months; race B came from a culture procured from the University of Pennsylvania; race C came from Powers and Powers, Nebraska; race D, was a wild race recently obtained from a pond in the neighborhood of the college laboratory.

A comparative study of pure lines from these four races was carried on with the addition of yeast both whole and ground to the food substance. Two drops each were used. Malted milk remained the basic food substance.

TABLE V.

A. LABORATORY CULTURE IN SPRING WATER. Daily Division Average For Six Day Periods.

Period.	Control.	Yeast, Whole.	Control.	Yeast, Ground.
1	16	11.5	9.5	11
2	10	10	9.5	13.5
Total	26	21.5	19	24.5
Average	2.6	1.78	1.41	2.03

These individuals had previously undergone a period of starvation living in the laboratory under adverse conditions. Here the malted milk alone seemed more favorable to growth without the addition of whole yeast. It will be observed, however, that when ground yeast was added that there was a distinctly marked increase in the division rate.

TABLE VI.

B. LABORATORY CULTURE IN HAY INFUSION. Daily Division Average For Six Day Periods.

Period.	Control.	Yeast, Whole.	Control.	Yeast, Ground.
1	12.5	10	10.5	10
2	13	10.5	8.5	14
Total	25.5	20.5	19	24
Average	2.11	1.7	1.6	2

The same result may be seen as in Table V., where the *Paramecia* had been in hay infusion.

TABLE VII.

C. MIXED CULTURE, POWERS AND POWERS. Daily Division Averaged for Six Day Periods.

Period.	Control.	Yeast, Whole.	Control.	Yeast, Ground.
1	6.5	7	10	10.5
2	7	7	6	3
Total	13.5	14	16	13.5
Average	1.11	1.16	1.33	1.11

This was a rich culture containing many infusoria, and a high percentage of bacteria. In these individuals the rate showed little increase above the average.

TABLE VIII.

D. WILD RACE. Daily Division Averaged For Six Day Periods.

Period.	Control.	Yeast, Whole.	Control.	Yeast, Ground.
1	9	5	11	7.5
2	12	2.5	8	1.5
Total	21	7.5	19	9
Average	1.75	.61	1.14	.75

The wild race of *Paramecia* had to undergo the greatest change. It thrived as the others on the rich diet of malted milk, but the experimental lines decreased exceedingly in division rate.

In the comparative study of these races it must be concluded that, (1) yeast stimulates growth as indicated by the rate of division, and (2) ground yeast is more favorable as food than whole yeast.

The physiological condition of the individual must be taken into consideration. For example in races A and B there was a higher rate of division than in races C and D. The latter ones had been in rich cultures before they were subjected to the experiment. Such a series also reveals the great variation in the rate for different individuals under the control conditions. For example, in Table V the variation of the control is 4.5, in Table VI., 3.2, in Table VII., 1.3 and Table VIII., 0.34. Although every effort was made to keep the conditions the same there is evidence of great variation.

In conclusion, the experiments performed with food hormones indicate (1) that potato extract will serve as a food but not as a growth accelerator, (2) that yeast extract serves as a food and stimulates growth, and (3) that the physiological condition of the individual plays a large part in determining the rate of division under experimental conditions. Results indicate that when food supply has been reduced a change of diet causes rapid increase in rate. Where food has been plentiful the rate of division has not been greatly increased.

2. Experiments with Glandular Diet.

Shumway (1917) has recently made a series of experiments with gland extracts. His controls were kept in a suspension of hay infusion. When adrenalin and pituitary extract were added these lines divided more than twice as rapidly as the control and with very little difference between them. In another experiment he determined to try what he termed a more crucial test,—*i. e.*, keeping the experimental lines in gland suspension alone without the basic food substance of hay infusion. Both lines died after a few days treatment.

For the following experiments a wild race of *Paramecia* was collected in January, 1918. Individuals descended from one original animal were put in hay infusion. On February 9, these *Paramecia* as control lines, were isolated in depression slides in a few drops of hay infusion and put in the moist chamber. Within twenty-four hours the sister *Paramecia* were isolated and the experimental lines established. The individuals were fed on solutions made from the tablets prepared in the laboratory of Armour & Co. The pituitary tablets were made from the desiccated gland of an ox and suprarenal from the desiccated gland of a sheep. The strength of the solution of the pituitary gland was .35 per cent., that of the suprarenal .36 per cent., being slightly stronger.

The experiments as recorded in Table IX. and Table X. were carried on for five weeks. With the pituitary solutions three proportions of food were used—first, control line two drops of hay infusion, experimental line one drop of hay infusion and one drop pituitary, second, control line three drops of hay infusion,

experimental line one drop of hay infusion and two drops of pituitary; third, control line four drops of hay infusion,—experimental line one drop of hay infusion and three drops of pituitary.

TABLE IX.

PITUITARY AND HAY INFUSION. Daily Division Average For Six-Day Periods.

Period.	Control.	Pituitary, One Drop.	Control.	Pituitary, Two Drops.	Control.	Pituitary, Three Drops.
1	.5	.66	.83	1.08	.5	.83
2	.5	1.0	.5	1.2	.92	1.0
3	.0	1.0	.83	.5	.66	1.3
4	.33	.75	.83	.5	1.2	.0
5	.5	1.0	.0	.25	.0	.33
Total	1.83	4.41	2.99	3.53	3.28	3.46
Average	.36	.88	.59	.65	.65	.69

There is an increase in division rate of experimental over control lines, but the amount of increase diminishes with an increasing amount of pituitary as food.

With the suprarenal two proportions of food were used;—first, control line two drops of hay infusion, experimental line one drop hay infusion, and one drop of suprarenal; second, control line three drops of hay infusion, experimental line one drop of hay infusion and two drops of suprarenal.

TABLE X.

SUPRARENAL AND HAY INFUSION. Daily Division Averaged For Six-Day Periods.

Period.	Control.	Suprarenal, One Drop	Control.	Suprarenal, Two Drops.
1	.8	1.0	1.5	1.1
2	.75	1.6	.66	1.1
3	1	.58	.0	1.0
4	1	.83	.66	1.2
5	.5	.66	.25	.41
Total	4.05	4.67	3.07	4.81
Average	.72	.943	.76	.96

These results are more constant than those obtained by feeding pituitary solution. There is a slight but decided increase over control lines.

The control of the experimental line containing two drops of suprarenal died and it was necessary to supply from stock which was kept in hay infusion.

A series of experiments was tried with mixtures of pituitary and suprarenal solutions in the basic food substance. The results indicate that the rate of division is greatly reduced. Sometimes the *Paramecia* would exist for seven days without dividing.

At this point in the work the control culture medium was changed from hay infusion to .2 per cent. malted milk. Another pedigreed race was started which had been in malted milk for several months. The solutions used were in two proportions—first, control line two drops of malted milk, experimental line one drop of malted milk, one drop of pituitary; second, control line three drops of malted milk, experimental line one drop of malted milk, and two drops of pituitary.

TABLE XI.

PITUITARY AND MALTED MILK SOLUTIONS. Daily Division Averaged for Nine Days.

Period.	Control.	Suprarenal, One Drop.	Control.	Suprarenal, Two Drops.
I	.89	0	1.1	0

In both experimental lines the *Paramecia* died after swimming about normally for five days without dividing. In no way can these results be correlated with the previous similar experiment where the basic food was hay infusion.

Solutions of suprarenal were the same as those above for pituitary.

TABLE XII.

SUPRARENAL AND MALTED MILK SOLUTION. Daily Division Average for Nine Days.

Period.	Control.	Suprarenal, One Drop.	Control.	Suprarenal, Two Drops.
I	.83	1	.77	1.1

There is an increase in division rate when Paramecia are fed suprarenal solution whether the basic fluid is hay infusion or malted milk.

Conclusions.

As a food hormone, potato extract apparently has little effect on the division rate of *Paramecium*. The influence of yeast is evident as exemplified in Tables III. and IV., *i. e.*, it increases the division rate.

In the comparative study of the four races the influence of yeast is not evident. Both control and experimental lines respond with increased division rate if the Paramecia have previously been in a starved condition. If food has been plentiful the rich diet of malted milk and yeast seems to have little effect.

Contrasting results were obtained with pituitary solution added to the hay infusion and malted milk basic substances. Suprarenal extract added to the basic fluid in which the *Paramecia* lived, caused an increase in the rate of division.

This experiment is to be considered as an indicator only. It was undertaken with the point in mind that if these solutions influenced the division rate of *Paramecia* a more extensive work would follow, *i. e.*, the bacterial content would be taken into consideration. It has been a generally accepted fact that the normal diet of *Paramecium* is composed of various kinds of bacteria and that in their absence growth does not take place, and even maintenance is impossible. In 1908 Jennings said, "To make the conditions of existence the same, it is not sufficient to attend merely to the basic fluid, the bacteria must also be the same."

Hargitt and Fray (1917) elaborated on previous methods of determining the bacterial content and they proved that *Paramecium* can live in media which have undergone sterilization provided at least one sort of bacteria remains in the culture.

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A FRESHWATER ANAËROBIC CILIATE.

CHANCEY JUDAY.

Investigations on a considerable number of Wisconsin lakes have shown that, in many of them, more or less of the lower stratum, or hypolimnion, and the muddy ooze at the bottom possess no free oxygen for a certain time during the summer stagnation period (Birge and Juday, '11). This time varies from three or four weeks in some lakes to as many months in others. The dissolved oxygen disappears gradually from this stratum as the season advances and when it reaches a minimal amount the various organisms occupying this region respond to the change either by migrating or by adapting themselves to the new conditions.

When the minimum is reached for plankton crustacea they rise to a higher level where oxygen is more abundant and when this gas becomes too scarce for some of the bottom dwelling insect larvæ they migrate to the shallower water. The majority of the forms, however, are able to remain here even in the absence of free oxygen. To this latter group belong representatives of at least seventeen genera of protozoa, including rhizopods, flagellates and ciliates, but chiefly ciliates, and a number of higher forms, such as insect larvæ, an ostracod, worms, and a bivalve mollusk. These forms appear to thrive as well in water which contains no free oxygen as in water which is well aërated, thus being facultative anaërobes (Juday, '08).

While making a study of the centrifuge plankton of Lake Mendota a ciliate was found in the lower stratum of water which appears to have gone beyond the facultative stage so that it has become substantially a true anaërobe. This ciliate agrees very closely in shape and structure with Fig. 126, Plate X., of Conn's "Protozoa of the Fresh Waters of Connecticut," which this author "with hesitation" places provisionally in the genus *Enchelys*. It has uniform ciliation, a rather pointed, obliquely truncate anterior end, and a rounded posterior extremity. Frequently

the anterior third of the organism appears to be filled with minute granules which give this portion a somewhat darker color. The maximum length ranges from 35 μ to 40 μ and the greatest width from 15 μ to 17 μ.

This ciliate was found each season from 1914 to 1917 inclusive, but it was most abundant in the former year and scarcest in the latter. The accompanying table shows its vertical distribution in four sets of observations which are typical of the entire series. This table also shows the relation of the organism to temperature, dissolved oxygen, and free carbon dioxide. The observations were made at a regular station where the lake has a depth of 23.5 meters.

TABLE I.

THIS TABLE SHOWS THE VERTICAL DISTRIBUTION OF THE CILIATE AND ITS RELATION TO TEMPERATURE, DISSOLVED OXYGEN AND FREE CARBON DIOXIDE.

Date.	Depth in Meters.	Number of Organisms per Liter.	Temperature, Degrees C.	Oxygen, c.c. per Liter.	Carbon Dioxide, c.c. per Liter.
October 10, 1914.....	14	0	17.4	1.67	2.50
	15	90,700	17.0	0.14	2.83
	16	58,900	16.3	0.00	3.92
	17	27,200	14.5	0.00	4.58
	18	0	13.8	0.00	5.14
October 21, 1914.....	17	0	15.2	0.60	3.53
	18	68,000	15.1	0.00	5.50
	19	68,000	14.6	0.00	7.92
	20	22,700	14.2	0.00	9.00
	21	9,100	13.3	0.00	9.24
	22.5	9,100	12.6	0.00	11.00
September 22, 1915.....	15	0	16.9	0.40	2.40
	16	27,200	15.9	0.00	5.67
	17	27,200	15.4	0.00	
	18	6,800	15.2	0.00	7.14
	20	0		0.00	
September 20, 1916.....	15	0	17.0	3.08	3.54
	16	13,600	14.8	0.00	7.33
	17	6,800	14.4	0.00	8.08
	18	6,800	14.0	0.00	8.84
	20	0	13.8	0.00	9.45

In 1914 regular observations were not begun until the first of September and this ciliate was first noted on September 16. The number rose to a maximum of 95,200 individuals per liter of water at a depth of 16 meters on September 29. From the latter date until October 21 the organism was abundant, but by October 23 the number had decreased 70 per cent., and by October

27 it had entirely disappeared. The autumnal overturn took place between the last two dates, with the consequent aëration of the water at all depths, and this event was followed by the prompt disappearance of the ciliate.

In 1915 this organism was found in small numbers in four sets of observations between July 12 and July 22. The stratum of water which it occupied at this time contained from 0.10 c.c. to 0.40 c.c. of dissolved oxygen per liter. Following the latter date it was not found again until August 19 and August 23 when a small number was noted in a stratum of water which contained no dissolved oxygen. It appeared regularly in catches made during September, the largest number being found during the latter part of this month. It disappeared between September 29 and October 6, the autumnal overturn and aëration of the lower water having taken place in the meantime.

In 1916 this ciliate was noted twice in July, but it did not appear again until September 18, when it was obtained during the following ten days. In 1917 it was found only in September and then only in small numbers.

This protozoan showed a downward movement each autumn which was correlated with the descent of the thermocline or mesolimnion, the organism keeping just below the stratum of aërated water. Thus in the table above it will be noted that the stratum occupied on October 21, 1914, was three meters deeper than that of October 10. This descent continued until the bottom was reached and then the ciliate disappeared promptly, usually within two days, after the bottom water became aërated. In the laboratory it lived in water that had been partially aërated only for a period of about twenty-four hours.

As indicated in the table there did not seem to be any correlation between the vertical distribution of this ciliate and the temperature of the water or the amount of free carbon dioxide, but there was a definite correlation with the lack of dissolved oxygen, the stratum occupied having at the most only a minimal amount of this gas and most frequently none at all. This fact and the further fact that it disappeared so promptly when the water became well aërated seem to warrant the conclusion that it is substantially an anaërobic ciliate.

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NOTE ON ANIMAL DISTRIBUTION FOLLOWING A HARD WINTER.

W. C. ALLEE.

LAKE FOREST COLLEGE.

For the past six summers I have been studying the distribution of littoral invertebrates near Woods Hole, Mass., in connection with a teaching appointment in the invertebrate course in the Marine Biological Laboratory. The work has been largely done in connection with the class trips which give a good opportunity for general collecting in that each of the six or seven instructors has from six to eight students working with him in the field. As a result of the class collecting during this period, there are over 190 species of invertebrates listed in the class catalogue that have been taken alive on student excursions.

Something of the thoroughness of the collecting may be seen from the fact that this work has added two new species to the faunal catalogue of this well-studied region: In 1915 Dr. T. S. Painter took a living specimen of *Arca ponderosa* (Say) in dredging off Naushon in Vineyard Sound. The animal was taken on the sand dollar grounds off Tarpaulin Cove in about six fathoms of water. Regarding this species the Biological Survey[1] says: "Verrill has expressed doubts as to whether *Arca ponderosa* lives in this region since no living specimen has been taken north of Cape Hatteras. Mr. G. M. Gray likewise reports that he has never taken this species alive. Dr. Dall informs us, however, that the National Museum contains a fresh valve, retaining the epidermis, taken in Vineyard Sound in 1870; and Mr. C. M. Johnson reports that he has found more than one shell of this species still bearing evident traces of the hinge ligament and epidermis, on a beach near Chatham Light." The extensive dredgings of the survey yielded only a few shells. The range is usually given as extending from Provincetown to Yucatan.

[1] Biological Survey of the Waters of Woods Hole and Vicinity by Francis B. Sumner, Raymond C. Osborn, and Leon J. Cole. Bull. Bureau of Fisheries, Vol. 31. Two parts.

Miss Eleanor Bach in 1916 found a specimen of *Halicystus auricula* (Clark) attached to eel grass in low water near the old Vineyard Haven wharf where it had apparently been washed up with algæ. This is a northward ranging species with a range from Cape Cod to Greenland.

A slight amount of preliminary collecting in the latter part of June, 1918, showed that the fauna was noticeably changed from the preceding year and that the change was decidedly greater than the usual yearly fluctuation. The preceding winter had been unusually severe and this offered an hypothesis for the cause of the unusual changes in the abundance of some animals.

The water temperatures have been taken for a number of

TABLE I.

Showing the Temperatures at Noon off the U. S. Fish Commission Wharf at Woods Hole, for the Four Winter Months of 1916–17 and 1917–18.

1916–17.					1917–18.			
Dec.	Jan.	Feb.	Mar.		Dec.	Jan.	Feb.	Mar.
46.5	32	33	32.5	1	41	29.5	30	32
45	33.5	32	32.5	2	40.5	29.5	30	32
44.5	34.5	30	32	3	39	30	30	32
45	34.5	30	32	4	39.5	29.5	30	31.5
46.5	34.5	30	32	5	39.5	30	30	32
46	35.5	30	32	6	39	30	30	32.5
44.5	35.5	30.5	32.5	7	38.5	31	30	32
44.5	35	31	33.5	8	37	30.5	30	31.5
44.5	36	31.5	34	9	38	30	30	31.5
44	36.5	31	33.5	10	36	30.5	30	32
43	36	31.5	33	11	35	31	30	31.5
44	33	30	35.5	12	34.5	31	30	31.5
42.5	33.5	30	33.5	13	34	30	31	32
42	35	30	34	14	34.5	30	31	31
41	33.5	30	34.5	15	33	30	31.5	31.5
39.5	33.5	31	34.5	16	33	30.5	31.5	30.5
39.5	32.5	31	34.5	17	33	30	31.5	30.5
38	33	31	35.5	18	33.5	30	31	31.5
37	32.5	31	34	19	33	30.5	31.5	32.5
36	32.5	31.5	34	20	33.5	30	32	32.5
37	32.5	31	34	21	34.5	30	31	33
37	33	31	34.5	22	34.5	30.5	31	32.5
37	32.5	30.5	34	23	33	30	31	34
36	32.5	31.5	35.5	24	33	30	31	33
36.5	32.5	31.5	36	25	34.5	30.5	31	34
35	32	32	37.5	26	33	30.5	32	34
35	31	32	37.5	27	32.5	30.5	31	34.5
36.5	31	31	38.5	28	32.5	30.5	32	34.5
35	31.5		38	29	31	30		36
34	33		38	30	29.5	30		36
32.5	32.5		39	31	29.5	28		36.5

years off the wharf of the U. S. Fish Commission at Woods Hole. The manuscript notes of the daily readings for the winters of 1916–17 and 1917–18 were made accessible through the kindness of Superintendent W. H. Thomas. The noon temperatures for the months of December, January, February and March of these years are given in Table I. From December 29, 1917, to March 19, 1918, a period of 80 days, the noon temperature did not rise above freezing, except on March 6, when it reached 32.5° F.

These temperatures are summarized in Table II., together with a similar summary for the period of 1902–06 taken from

TABLE II.

Showing Mean, Maximum and Minimum Noon Temperatures off the Fish Commission Wharf at Woods Hole, Mass.

Period.	December.	January.	February.	March.
1902–1906. Mean.....	37.2	32.3	31.0	35.64
Minimum.....	31.5	28.0[1]	28.0[1]	29.5
Maximum.....	47.5	39.5	37	44
1916–1917. Mean.....	39.9	33.4	30.9	34.3
Minimum.....	32.5	31	30	32
Maximum.....	46.5	36.5	33	39
1917–1918. Mean.....	35.1	30.1	30.75	32.7
Minimum.....	29.5	28[1]	30	30.5
Maximum.....	46.5	31	32	36.5

[1]Inexact; below the freezing point of water.

the Biological Survey. It will be noted that the mean temperature for each of the months considered is less than for the corresponding month of the preceding year or of the five-year interval; that February, the coldest month, is only slightly below that of the other years, but that the other months are all decidedly lower and that the cold weather continued practically through March.

The mean noon temperature for the 121 days listed, in the five-year period from 1902–06 was 34.4 degrees. In 1916–17 there was a mean temperature of 35.5 degrees during this winter period, while for 1917–18 the mean temperature was only 33.3 degrees. This gives a temperature deficit of 1.1 degrees per day when compared with the longer period, and of 2.2 degrees when compared with the preceding winter.

The effect of an usually cold winter should be most marked

upon animals near the northern limit of their geographical range. The result of an inquiry as to whether or not such animals were affected is shown in the list given as Table III. The list is made from the class list of invertebrates whose distribution, as shown in Pratt's Manual of Common Invertebrate Animals, is that of southern ranging animals. By "southern ranging" is meant that the known geographical range extends twice as far south as north of the point under consideration. The frequency data is based principally on my own experience in the field assisted by that of Mr. J. S. Kostir, of Ohio State University, who has collected over the same ground for the last two summers.

TABLE III.

A List of Species, that are South Ranging according to Pratt. Taken by the Invertebrate Class of the Marine Biological Laboratory. The Comments in the Last Column Give the Frequency Data as Compared with the Preceding Summer.

Species	Range	Frequency
Porifera:		
Cliona celata (Grant)	S. Ca.–Maine.	Fewer.
Chalina arbuscula (Ver.)	N. Ca.–Cape Cod.	Fewer.
Microciona prolifera (Ellis and Solander)	S. Ca.[2]–Cape Cod.	Fewer.
Coelenterata:		
Pennaria tiarella (Ayres)	Fla.–Maine.	Fewer.
Schizotricha tenella (Ver.)	Beaufort,[1] N. C.–Marthas Vineyard.	No change.
Dactylometra quinquecirrha (Desor)	Tropics–Vineyard Sound.	Comparison unfair.
Sagartia luciæ (Ver.)	Florida[2]–Cape Cod.	Fewer.
Sagartia leucolena (Ver.)	N. Ca.—Cape Cod.	More.
Eleoactis producta (And.)	S. Ca.—Cape Cod.	Fewer.
Astrangia danæ (Ag.)	Fla.—Cape Cod.	Fewer.
Nemertini:		
Micrurl leidyi (Ver.)	N. J.—Cape Ann.	Fewer.
Cerebratulus lacteus (Leidy)	Fla.—Maine.	Fewer.
Echinoderma:.		
Asterias forbesi (Desor)	Common south of Cape Cod Rare north to Maine.	More small.
Ophioderma brevispina (Say)	South from Cape Cod.	Fewer.
Arbacia punctulata (Gray)	Yucatan[2]—Cape Cod.	Fewer.
Thyone briareus (Lesueur)	South from Vineyard Sound.	No change.
Annelida:		
Sthenelais leidyi (Quatr.)	N. Ca.—Mass. Bay.	No change.

Podarke obscura (Ver.)	G. of Mexico—Cape Cod.	Fewer.
Nereis limbata (Ehlers)	S. Ca.—Maine.	No change.
Platynereis megalops (Ver.)	Beaufort, N. C.—Cape Cod.	Comparison unfair.
Diopatra cuprea (Bosc.)	S. Ca.—Cape Cod.	No change.
Lumbrinereis tenuis (Ver.)	Va.—Mass.	No change.
Arabella opalina (Ver.)	West Indies—Maine.	No change.
Glycera americana (Leidy)	S. Ca.—Cape Cod.	No change.
Scoloplos robustus (Ver.)	N. Ca.—Cape Cod.	No change.
Chætopterus pergamentaceus (Cuv.)	N. Ca.—Cape Cod.	No change.
Cirratulus grandis (Ver.)	Va.—Cape Cod.	No change.
Pista palmata (Ver.)	Va.[1]—Vineyard Sound.	Comparison unfair.
Amphritrite ornata (Leidy)	N. Ca.—Cape Cod.	No change.
Polycirrus eximeus Leidy)	N. Ca.—Cape Cod.	No change.
Pectinaria gouldi (Ver.)	N. Ca.—Maine.	No change.
Maldane urceolata (Leidy)	N. Ca.—Cape Cod.	No change.
Arenicola cristata (Stimp.)	Fla.—Cape Cod.	No change.
Trophonia affinis (Leidy)	N. J.—Vineyard Sound.	Comparison unfair.
Clymenella torquata (Leidy)	N. Ca.—Bay of Fundy.	No change.
Sabella microphthalmia (Ver.)	N. Ca.—Cape Cod.	Fewer.
Hydroides hexagonus (Bosc.)	Fla.—Cape Cod.	Fewer.
Sabellaria vulgaris (Ver.)	N. Ca.—Cape Cod.	Fewer.
Bryozoa:		
Bugula turrita (Desor)	N. Ca.—Casco Bay.	No change.
Schizoporella unicornis (Johnston)	S. Ca.[2]—Mass. Bay.	Fewer.
Arthropoda:		
Balanus eburneus (Gould)	W. Indies—Mass. Bay.	No change.
Orchestria agilis (Smith)	Ranges south to Florida.	No change
Talorchestria longicornis (Say)	N. J.—Cape Cod.	No change.
Haustorius arenarius (Slabber)	Ga.—Cape Cod.	No change.
Caprella geometrica (Say)	Va.—Cape Cod	Fewer.
Sphæroma quadridentatum (Say)	Fla.—Cape Cod.	Fewer.
Chiridotea cæca (Say)	Fla.—Nova Scotia.	Comparison unfair.
Palæmonetes vulgaris (Say)	Fla.—Mass.	No change.
Pagurus longicarpus (Say)	S. C.—Maine.	No change.
Pagurus pollicaris (Say)	Fla.—Maine.	No change.
Pagurus pollicaris (Say)	Fla.—Maine.	No change.
Hippa talpoida (Say)	Fla.[1]—Cape Cod.	Fewer adult.
Libinia emarginata (Leach)	Fla. Maine.	Fewer.
Libinia dubia (Milne-Ed.)	Fla.—Cape Cod.	More.
Panopeus sayi (Smith)	Fla.—Mass.	No change.
Callinectes sapidis (Rath.)	La.—Cape Cod.	Fewer.
Ovalipes ocelatus (Herbst)	G. of Mex.—Cape Cod.	Fewer.
Carcinides maenas (L.)	N. J. —Cape Cod.	Fewer.
Pinnotheres maculatus (Say)	S. C.—Cape Cod.	No change.

Uca pugilator (Bosc.).....	Fla.—Cape Cod.	No change.
Limulus polyphemus (L.).....	Fla.—Nova Scotia.	More.
Tanystylum orbiculare (Wilson).....	Va.[1]—Martha's Vineyard.	Comparison unfair.
Mollusca:		
Chætopleura apiculata (Say).....	Fla.—Cape Cod.	No change.
Melampus lineatus (Say).....	Tex.—N. Eng.	No change.
Natica duplicata (Say).....	Mex.—Mass. Bay.	No change.
Crepidula fornicata (L.).....	S. Am.—Nova Scotia.	No change.
Crepidula convexa (Say).....	Fla.—Nova Scotia.	No change.
Crepidula plana (Say).....	Tex.—Maine.	No change.
Bittium alternatum (Say).....	N. J.—Mass. Bay.	No change.
Cerithiopsis greeni (Adams).....	Tex.—Mass. Bay.	No change.
Eupleura caudata (Say).....	Fla.—Cape Cod.	Comparison unfair.
Urosalpinx cineros (Say).....	Fla.—Mass. Bay.	No change.
Columbella lunata (Say).....	Fla.—Mass. Bay.	No change.
Columbella avara (Say).....	Fla.—Cape Cod.	Fewer.
Nassa trivittata (Say).....	Fla.—G. of St. Law.	No change.
Nassa obsoleta (Say).....	W. Fla.—G. of St. Law.	No change.
Busycon carica (L.).....	G. of Mex.—Cape Cod.	Fewer.
Busycon canaliculatum (L.).....	G. of Mex.—Cape Cod.	Fewer.
Anomia ephippium (L.).....	W. Ind.—Nova Scotia.	Fewer.
Arca pexata (Say).....	Fla.—Maine.	Fewer.
Arca ponderosa (Say).....	Fla.—Cape Cod.	Comparison unfair.
Arca transversa (Say).....	Fla.—Cape Cod.	Fewer.
Ostrea virginica (Gmelin).....	G. of Mex.—Mass.	Fewer.
Pecten irradians (Lam.).....	Tex.—Cape Cod.	Fewer adult.
Tellina tenera (Say).....	W. Fla.—G. of St. Law.	No change.
Cumingia tellinoides (Conrad).....	Fla.—Cape Cod.	No change.
Venus mercenaria (L.).....	Tex.—G. of St. Law.	No change.
Petricola pholadiformis (Lam.).....	Tex.—G. of St. Law.	Fewer.
Laevicardium mortoni (Conrad).....	W. Fla.—Nova Scotia.	Fewer.
Clidiophora trilineata (Say).....	Tex.—Nova Scotia.	Comparison unfair.
Chordata:		
Dolichoglossus kowalevskyi (A. Agassiz).....	Beaufort, N. C.—Mass. Bay.	No change.
Styela partita (Stimp.).....	N. C.—Mass. Bay.	Fewer.
Molgula manhattanensis (DeKay)...	N. C.—Casco Bay.	Fewer.
Perophora viridis (Ver.).....	Bermuda[1]—Vineyard Sound.	Fewer.
Amaroucium pellucidum (Leidy)....	N. C.—Vineyard Sound.	No change.
Amaroucium stellatum (Ver.).....	N. C.—Cape Cod and north.	No change.

Summary: Fewer, 36; no change, 45; more, 5; unfair comparison, 9.

[1] Range taken from Biological Survey.

[2] Discussed in text following.

In order to check up on this data I asked Mr. G. M. Gray for a list of the forms that had proven difficult to obtain during the 1918 season. I did not ask him to refer to his records because only those animals that had been so difficult to obtain that they were well fixed in his mind were wanted. This group of animals with their distribution is given as Table IV. An inspection of the table will show that in each case the animals are southern ranging forms with Woods Hole near the northern limit of their range.

TABLE IV.

Listing the animals which Mr. G. M. Gray found to be especially difficult to obtain in the summer of 1918 as compared with his experience in other years.

Sagartia luciæ (Ver.)	Fla.—Mass. Bay.
Astrangia danæ (Ag.)	Fla.—Cape Cod.
Arbacia punctulata (Gray)	Yucutan—Cape Cod.
Hippa talpoida (Say)	Fla.—Cape Cod.
Callinectes sapidus (Rath)	La.—Cape Cod.
Styela partita (Stimp.)	N. C.—Mass. Bay.
Perophora viridis (Ver.)	Bermuda—Vineyard Sound.

Arbacia punctulata had been particularly abundant in 1917. Small specimens were taken from rocks at Kettle Cove, at the Buzzard Bay entrance to Northwest Gutter, near Quissett and at North Falmouth. In all these places they came up almost to the low tide limit. In the deeper water they had again appeared in numbers on what the collecting crew called "the old *Arbacia* grounds" off Nobska Light. In 1918 none were taken by the class except in dredging in Vineyard Sound off Tarpaulin Cove, and then only a few small individuals. In fact during all the summer, in place of the usual abundance of large specimens, Mr. Gray was able to furnish only relatively few small *Arbacia*.

Sagartia luciæ, usually the most abundant actinian in the Woods Hole region, was almost wanting at the beginning of the summer in 1918. In only one place visited throughout the summer was this small anemone present in anything like its usual abundance. This was at Kettle Cove, where in the protected tide pools, the rocks were covered in about their normal fashion. On the exposed parts of Kettle Cove, as everywhere else, the rocks were practically bare. Pratt gives the distribution of this species as Long Island Sound to Massachusetts Bay and further

north. The Biological Survey says that it first appeared in New Haven in 1892 and at Woods Hole in 1898. Professor Verrill believed that this *Sagartia* was introduced near New Haven on oysters from the south. Professor Parker states that it is a southern ranging species.

One more particular instance of a marked decrease in abundance was that of the red sponge, *Microcione prolifera*. In the early part of the season this sponge was almost absent from the wharf piles. Later they became more abundant.

In a number of instances the cold weather killed off the animals living in shallow water and left only those in deeper water. This tendency has already been mentioned in the case of *Arbacia*. It was also marked with the encrusting bryozoa as, *Schizoporella unicornis*.

In Table III. the data concerning *Platynereis* is listed as being insufficient to furnish a fair comparison. As a matter of fact none were taken this year but usually the number taken in the class collecting has been small and it is possible that we overlooked them this year. However, Dr. E. E. Just, who has been collecting swarming annelids for some years, reported that up to August 1 he had taken no *Platynereis* in his night collections although usually they are abundant by that time. *Autolytus cornutus* (A. Agassiz) was also lacking in his collections at that date although normally present. Unlike the other species this annelid is northerly ranging. It is found from New Jersey to the Bay of Fundy. Dr. Just also reported that the cold spring had affected the periodicity[1] of the early runs of *Nereis limbata* in a way wholly comparable with other late springs.

It is to be expected that the negative effect of a cold winter would be more noticeable than its positive effect. However, two northerly ranging species were present in decidedly increased numbers in the collecting. The more noticeable of these was the sponge, *Leucosolenia botryoides* (Bow.). Pratt gives its range as from Martha's Vineyard to the Gulf of St. Lawrence. Usually we have taken it to some extent in the class collecting on wharf pilings, but this year it was very abundant there and was found

[1] Lillie, F. R., and Just, E. E., 1913, "Breeding Habits of the Heteronereis. Form of *Nereis limbata* at Woods Hole, Mass.," BIOL. BULL., 24, pp. 147–169.

in some abundance on rocks, such as those at the Buzzard Bay entrance to Northwest Gutter.

The blood starfish, *Henricia sanguinolenta* (O. F. Müller), distributed from Cape Hatteras to Greenland and reported as common north of Cape Cod, was decidedly more abundant this year than usual.

The effects of the unusually cold winter on the abundance of the animal life may be due to one or all of the following factors:

1. To the temperature deficit, regardless of the duration of the cold weather. In this case the deficit amounted to 133 degrees when the winter months were compared with those of the five year period of 1902–06.

2. To the long-continued cold weather (practically 80 days at or below freezing).

3. To the fact that the cold weather began relatively early, or that it lasted relatively late, without regard to the temperature in the mid-winter interval.

Of these possibilities, I am inclined to regard the second and third as furnishing the cause for the observed change on the animals, with the fact that the cold weather lasted well through March as the most important factor. This is supported by the February temperatures, which were practically the same for the three periods listed, so the temperature for this month could have made little difference, and by general experience that a "late spring" gives some approach to the conditions found this summer.

A SIMPLE METHOD FOR MEASURING CARBON DIOXIDE PRODUCED BY SMALL ORGANISMS.[1]

E. J. LUND.

The suitable methods available at the present time for measuring the carbon dioxide produced by single cells such as eggs and small organisms weighing not over one or two grams are limited in number and usually only suitable for special types of material. Among the best methods for measuring or comparing small quantities of carbon dioxide produced by small organisms are those by Thunberg ('05) as modified by Winterstein ('13), and Krogh ('16). These are in turn based on the older and well known methods of Pettenkoffer and Petterson. Another method is that used by Warburg ('09) in his studies on sea urchin eggs and a more recent and apparently extremely sensitive method for the detection and estimation of very small quantities of CO_2 is that of Tashiro ('17). All of these methods may have their own advantages, depending upon the material and the nature of the problem. However, if many separate determinations of CO_2 are to be made at the same time, the apparatus must be duplicated or if not, the complexity of the procedure is increased greatly and thus becomes practically impossible.

In attempting to devise some simple method for measuring at the same time the small amounts of CO_2 produced by separate samples of *Paramecium*, or small invertebrates weighing one gram or less, the following procedure was adopted. Fig. 1 and the photograph, Fig. 2, show the arrangement. Two bottles of about two liters capacity, one containing $n/100$ HCl and the other a solution of $Ba(OH)_2$ of any concentration between $n/75$ and $n/150$, are provided with soda lime tubes and burettes. The solutions should be made up with CO_2 free distilled water as described in Treadwell and Hall ('15). A bottle of about 100 c.c. capacity, has a large glass stopper which fits the bottle very accurately and from which is suspended a small dish by

[1] From the Department of Animal Biology, University of Minnesota.

means of the hook and wire. The stender dish, Fig. 1, *d*, may either be suspended as shown or held rigidly by an appropriately shaped clamp of wire fixed to the stopper with cement.

The procedure in brief is as follows: One to five cubic centimeters of a suspension of *Paramecium* which have previously been gradually transferred to pure tap water from native hay infusion are placed in the dish, Fig. 1, *d*. Ten or fifteen cubic centimeters of the $n/100$ $Ba(OH)_2$ solution are run into the bottle

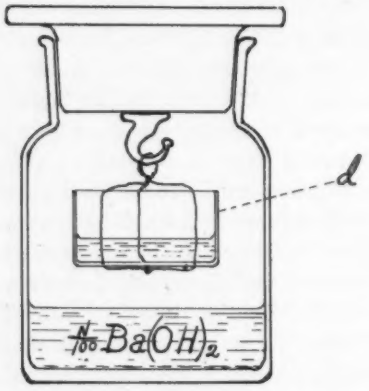

FIG. 1.

through a small hole in a cardboard disk which is placed over the mouth of the bottle shown in the photograph. The dish is then placed on the hook of the stopper and the bottle quickly stoppered as shown in Fig. 1. The ground glass stopper should be slightly greased and the bottle tightly stoppered. The CO_2 already in the air in the bottle and the CO_2 given off from the organisms in the suspended dish diffuses into the $Ba(OH)_2$ solution and quickly forms the insoluble white precipitate of $BaCO_3$. This absorption continues until no more $Ba(OH)_2$ remains if sufficient CO_2 is produced to neutralize all the free base, for the $BaCO_3$ is practically insoluble and hence the reaction goes practically to completion. At the end of the desired period, the length of which is determined by the rate of CO_2 production by the organisms and conditions of the experiment, the stopper is quickly removed and the bottle is again covered with

the cardboard disk. A drop of phenolphthalein is added through the hole in the cardboard disk and then the $Ba(OH)_2$ is rapidly titrated with a weak solution of HCl of known and appropriate concentration, say $n/100$. As control a similar bottle is set up in *exactly* the same way but *without* the animals. The $Ba(OH)_2$ is titrated at the end of the experiment, that is, at the same time as the free $Ba(OH)_2$ in the experimental bottle. The difference in the amounts of HCl necessary to neutralize the remaining free base in the bottles is equivalent to the quantity of CO_2 pro-

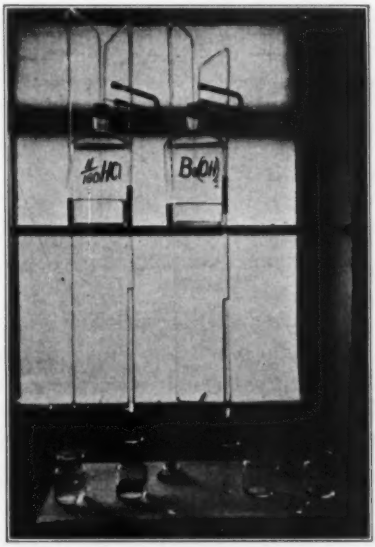

FIG. 2.

duced by the organisms during the period of the experiment. This description explains in brief the principle and procedure of the method, but the following sources of variation and error suggest themselves and will be considered in the same order as follows:

1. Temporary exposure to the CO_2 in the air of the open bottle containing the $Ba(OH)_2$ may result in absorption of varying amounts of CO_2 in different individual bottles. This variation

may be due to non-uniformity of manipulation and different CO_2 content of the air in the room. The greatest source of error comes from the CO_2 in the expired air of the experimenter and from burning flames in the room.

2. If the organisms are in water in the dish, Fig. 1, *d*, then the CO_2 dissolved in this water either as dissolved free CO_2 or possibly partly in the form of bicarbonate, may vary unless precautions are taken to control this source of variation of CO_2.

3. Another possible source of variation is the excretion of CO_2 by bacteria in the liquid in the dish, if this is for example a hay infusion containing *Paramecium* or from some other source depending upon the material experimented upon. This source of error is present and must be controlled in all other methods for CO_2 determination as well as in the one described here.

4. Finally perhaps the most important question is: how uniform, rapid and complete is the absorption of the CO_2 from the air in the bottle, by a $n/75$ to $n/150$ $Ba(OH)_2$ solution?

Repeated tests have shown that all of the above four sources of error can readily be either eliminated or controlled if the following precautions are taken.

The first source of error mentioned under number one above can be controlled and thus practically eliminated by *working in a well-ventilated room in front of an open window through which fresh air from the out doors is entering*, and *by covering the mouth of the bottle with a perforated cardboard disk during the addition of the $Ba(OH)_2$ at the beginning, and titration with HCl at the end of the experiment.* It is best to aërate the open bottle by holding or shaking it in the stream of air from the window just before covering it with cardboard disk and the rapid addition of the required amount of $Ba(OH)_2$ from the burette. The pasteboard disk should be held tightly over the bottle during all the manipulation and only removed just before the stopper with the small dish is inserted. A similar procedure must be carried out at the end of the experiment during titration of the remaining free $Ba(OH)_2$ with HCl.

The current of air from the window carries the exhaled air of the experimenter away from the bottles and makes the CO_2 content of the air the same in the duplicate bottles.

Sufficiently uniform manipulation in different determinations is very easily attained as the results below will show. The amount of CO_2 contained in the air in the bottle is determined by titrating with $n/100$ HCl, 15 c.c. of $n/100$ $Ba(OH)_2$ in each of two bottles. One of these samples of $Ba(OH)_2$ is titrated at once and the other at the end of an hour. The difference in the amounts of HCl is equivalent to the CO_2 of the air in each of the bottles at the beginning. To increase the accuracy three such tests are made at the same time and the average of the three taken as the true value.

The following are typical examples of tests showing the degree of uniformity.

Three samples of 15 c.c. $Ba(OH)_2$ each when titrated at once using one drop of phenolphthalein took

12.65 c.c.
12.70 c.c.
12.65 c.c.

Average 12.66 c.c. $n/100$ HCl

At the end of 1 1/2 hours three other samples of 15 c.c. of the same $Ba(OH)_2$ solution in each, took

12.35 c.c.
12.32 c.c.
12.32 c.c.

Average 12.33 c.c. $n/100$ HCl.

The amount of CO_2 absorbed was therefore equivalent to the difference or .33 c.c. $n/100$ HCl.

Several days later another set of three samples of 10 c.c. each of $Ba(OH)_2$ solution titrated at once, took

8.32 c.c.
8.32 c.c.
8.35 c.c.

Average 8.33 c.c. $n/100$ HCl.

At the end of 2 1/2 hours three corresponding samples took

8.15
8.13
8.14

Average 8.14 $n/100$ HCl.

The amount of CO_2 absorbed from the air in the bottle and during the manipulation on this day was therefore equivalent to .19 c.c. $n/100$ HCl.

The above illustrates the following facts: (*a*) The manipulation described above permits of uniform titration of the free $Ba(OH)_2$. (*b*) The amounts of CO_2 absorbed in duplicate blank bottles after allowing time for absorption, are the same. (*c*) The CO_2 absorbed from the outdoor air during manipulation and from the volume of air enclosed in the bottle may vary from day to day with different conditions but is uniform over brief periods of time. It is therefore clear that whenever necessary, proper and adequate controls to determine this amount of CO_2 can readily be provided in experiments.

If the organisms are in water in the dish, Fig. 1, *d*, then another source of variation must be taken into account, viz., possible differences in amounts of CO_2 in the water in the dish. This may be made uniform by taking equal, and as small volumes as possible, of the medium in which the organisms live, and still further reduced to small and uniform amount by using tap water as a medium as for example with *Paramecium*, Lund ('18). That the variation exists and is quite definite is indicated in the following Table I.

TABLE I.

The native medium was tap water in which Paramecia had been living for several hours and from which they were removed with the centrifuge. Twenty c.c. $Ba(OH)_2$ added to each bottle.

Volume of Native Medium in each Dish.			
1 C.c.	2 C.c.	3 C.c.	None.
Titrated at End of 18 Hours.			Titrated at Beginning.
C.c. n/100 HCl.	C.c. n/100 HCl.	C.c. n/100 HCl.	C.c. n/100 HCl.
20.45	20.20	20.00	21.40
20.61	20.35	19.97	21.35
20.55	20.30	20.00	21.40
Average 20.53	20.26	19.99	21.36

With air breathing organisms this source of variation is of course absent. Escape of such organisms from the dish may be prevented by a wire gauze cap placed over the small dish.

In order to determine the rapidity and completeness of CO_2 absorption by the $Ba(OH)_2$, simple and critical tests were carried

out as follows. A known amount, 1 to 5 milligrams of Na_2CO_3 were weighed out in a small glass vial. This vial with the carbonate was placed in 2 c.c.–3 c.c. 50 per cent. H_2SO_4 in the dish, Fig. 1, *d*, and suspended in the bottle over the $Ba(OH)_2$. The vial was then tipped so as to allow the H_2SO_4 to decompose the carbonate, thus liberating all CO_2 from a weighed amount of Na_2CO_3. The rate and completeness of absorption of this liberated CO_2 was then tested by titrating the $Ba(OH)_2$ at different intervals of time after decomposition of the carbonate. A typical test was carried out as follows. Ten one milligram lots of Na_2CO_3 were weighed out in the glass vials as accurately as the balances and weights used would permit. One of the vials was placed in each bottle as described, over 10 c.c. $Ba(OH)_2$ solution. The time when the Na_2CO_3 was decomposed was noted and titrations made at different intervals thereafter. Controls to determine the amount of CO_2 absorbed from the air in the bottles accompanied the experiment. Results of such an experiment are given in Table II.

TABLE II.

Lots of 1 milligram Na_2CO_3 each, decomposed with 50 per cent. H_2SO_4 in the closed bottle. Ten c.c. of an approximately *n*/100 $Ba(OH)_2$ added to each bottle. Temperature 29° C.

Bottle.	Controls. No Na_2CO_3. Titrated with *n*/100 HCl at		One Milligram Na_2CO_3 Decomposed. Titrated with *n*/100 HCl at End of			
	Once.	End of 2¼ Hours.	15 Minutes.	30 Minutes.	1 Hour.	2¼ Hours.
1	8.40	8.15	6.42	6.42	6.40	6.37
2	8.38	8.17	6.35	6.50	6.34	6.45
3	8.35	8.13	6.62	6.57		
Average	8.38	8.15	6.46	6.49	6.37	6.41
c.c. *n*/100 HCl equivalent of CO_2 in air		.23				
Average c.c. *n*/100 HCl equivalent of CO_2 recovered from 1 mgm. Na_2CO_3			1.69	1.66	1.78	1.74
c.c. *n*/100 HCl equivalent of CO_2 expected						1.84

The absorption was evidently very rapid and nearly complete in less than thirty minutes to an hour. The average amount of CO_2 not recovered in this experiment equivalent to about 0.1

c.c. .0098*n* HCl is perhaps largely due to error in weighing such a small quantity as one milligram on the balances which were only sensitive to about 1/20 mgm. Considering the small amounts of CO_2 involved, the degree of accuracy obtainable is rather striking; the amount of CO_2 apparently not recovered in the above test being about 5 per cent. of the CO_2 liberated from 1 mgm. Na_2CO_3 or about .01 c.c. CO_2 at N.T.P. In another similar experiment using lots of 1 mgm. Na_2CO_3 about 5 per cent. *more* CO_2 was recovered than was expected from 1 mgm. of carbonate. This indicates that the error was perhaps largely one due to weighing. In one test in which was used 2 mgm. of carbonate all of the CO_2 was absorbed in less than $1^1/_2$ hours. Still another similar test to determine the rate of absorption of CO_2 from 5 mgm. of carbonate showed that at the end of fifteen minutes over 60 per cent.–70 per cent. of all the CO_2 was absorbed, and at the end of one hour all of the CO_2 was absorbed. To show that within the limits of error in weighing, the CO_2 in 2, 3 and 5 milligrams of carbonate can be practically completely recovered in relatively short periods of time by 15 c.c. weak solution of $Ba(OH)_2$ the results in Table III. are given.

TABLE III.

Bottle.	Amount of Na_2CO_3 Decomposed.		
	2 Mgms.	3 Mgms.	5 Mgms.
	Equivalent of CO_2 Recovered, in C.c. .0098*n* HCl.	Equivalent of CO_2 Recovered, in C.c. .0098*n* HCl.	Equivalent of CO_2 Recovered, in C.c. 0098*n* HCl.
1	3.84	5.45	8.88
2	3.79	5.77	8.83
3	3.95	4.86	8.80
4	3.67	5.16	8.75
5	3.75	5.29	
6	3.39	5.05	
Average	3.73	5.26	8.81
Average per milligram of Na_2CO_3	1.86	1.75	1.76
Expected per milligram Na_2CO_3	1.84		

The differences among the individual analyses were rather larger in this test than in some of the others. One and one half

to two hours were allowed for absorption of the CO_2 in the above test.

The applicability of the method to measurement of CO_2, like some other methods depends upon the assumption that no other volatile acids than CO_2 are excreted by the organisms.

The carbon dioxide production by Paramecium and various small invertebrates has been measured and the results show that the method is satisfactory. For example in practical tests on Paramecium it was found that twice the number of Paramecia always produced twice the amount of CO_2, other conditions being exactly the same in two experiments. When using Paramecium the method is of course brought to a most rigid test of its usefulness, since the amounts of CO_2 produced by 2 c.c.–4 c.c. of fairly concentrated Paramecium suspension in 8 to 24 hours are much smaller than those produced by an active animal weighing one gram. Success with the method depends to a considerable extent upon a proper appreciation of the rôle of the different factors described above and care in manipulation.

Among what appear to be the special advantages of the method are the following:

1. It is simple and direct.

2. Many determinations can be made in a short time when compared with other methods.

3. The range of adaptibility is considerable and the percentage of error of the method is relatively small when the small amounts of CO_2 which can be measured, is considered.

4. The apparatus is small and many bottles can be immersed in a water bath at the same time, hence temperature can be accurately controlled in many measurements carried out at the same time.

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THE QUESTION OF THE PHYLOGENETIC ORIGIN OF TERMITE CASTES.

CAROLINE BURLING THOMPSON[1] AND THOMAS ELLIOTT SNYDER.[2]

It has recently been shown by one of the authors (C. B. T.) that the ontogenetic origin of the castes of the termite *Reticulitermes flavipes* Kol., formerly *Leucotermes flavipes*, is due to intrinsic causes, and not to the extrinsic stimuli which have long been credited with formative, indeed almost creative powers. Grassi (1893–94) and his followers described the plastic, "undifferentiated," newly hatched nymphs, upon which the action of the stimuli of food, parasites, fraternal care, etc., wrought out the highly differentiated adult castes. In favor of Grassi's hypothesis is the fact, that, in most genera, the newly hatched termite nymphs, about one millimeter long, are externally all alike. On the other hand, Grassi's hypothesis is disproved by the recent observations that termite nymphs are already differentiated at the time of hatching (Bugnion, 1912–'13, Thompson, 1917).

Bugnion states that, at the time of hatching, the soldier (nasutus) caste of the termite *Eutermes lacustris* Bugn. is sharply differentiated from the other nymphs by external structural characters. He has not worked out the differentiation of the other castes, which is, of course, a simple matter of observation, but his general conclusion, based upon this and other investigations, is that the castes of *Eutermes lacustris* originate in the embryonic period, and that the cause of differentiation is deep seated, and probably analogous with the cause of sex (Bugnion, 1912, '13).

Thompson (1917) finds that in *Reticulitermes flavipes* the newly hatched nymphs are externally all alike, but are differentiated by internal structural characters into two clearly defined

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types, the reproductive and the worker-soldier types, which give rise, respectively, to the three adult reproductive castes and the two adult sterile castes. The two types of newly hatched nymphs are distinguishable by four structural characters: the

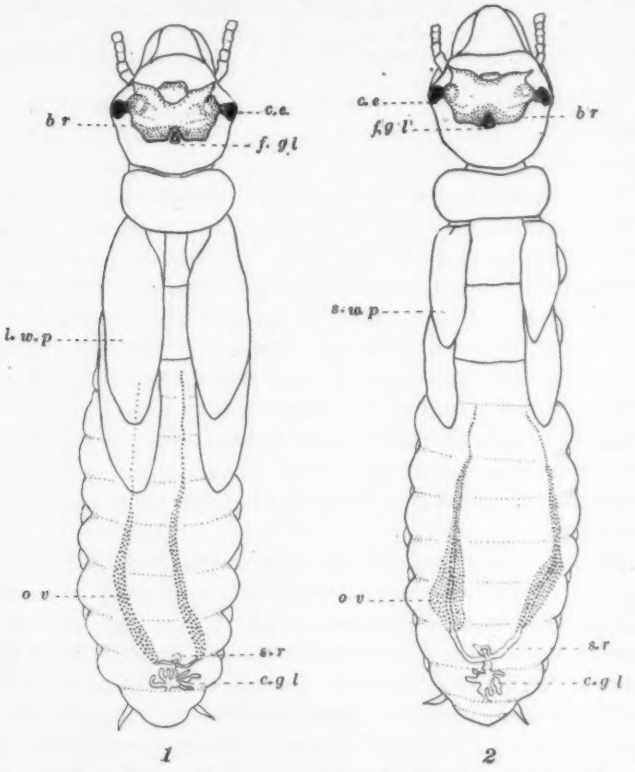

FIG. 1. *Reticulitermes flavipes*. Female nymph of the first form, drawn from a whole mount.

FIG. 2. *Reticulitermes flavipes*. Female nymph of the second form, drawn from a whole mount. The ovaries, *ov*, which in their later development, are of secondary size, are now more swollen than in the nymph of the first form, since the ova of the second form nymphs mature at an earlier age. For the relative size of the ovaries of mature queens of the first and second forms, compare Figs. 6 and 7. It should be noted that all the parts of the reproductive system are fully formed and connected in these two fertile nymphs.

Br, brain; *c.e*, compound eye; *c.gl*, colleterial gland; *f.gl*, frontal gland; *l.w.p*, long wing pad; *s.w.p*, short wing pad; *s.r*, seminal receptacle; *ov*, ovary. Oc. 6, obj. 32 mm., drawn at stage level. Reduced one third. Drawn by C. B. T.

bulk of the brain, the relative size of brain and head, the compound eyes, and the sex organs. When the young reproductive nymphs have attained a length of 1.3–1.4 mm. other structural differences are observable that further differentiate them into two kinds of individuals which later develop into two of the three adult reproductive castes, namely: adults of the first form, with long wings, and adults of the second form, with short, scale-like wing pads. The ontogeny of the third adult reproductive caste, without wing stubs or wing pads, is yet to be worked out in *R. flavipes*. At a later period in the ontogeny—body length about 3.75 mm.—the worker-soldier nymphs differentiate into two kinds of nymphs which develop into the two sterile adult castes, the workers and the soldiers.

As a result of the discovery that the ontogenetic cause of termite castes is intrinsic, two questions arise. First, can this intrinsic ontogenetic cause be determined by cytological means, *i. e.*, by examination of the phases of ovogenesis and spermatogenesis? Second, what is the phylogenetic origin of the termite castes? It is with this second question that the present paper deals.

The phylogenetic mode of origin of the castes of termites, could it be determined, might have an important bearing upon the general question of the evolution of species. The condition of polymorphism, or the existence of several structural forms within a species, indicates that the parent or ancestral form had a tendency to vary. Two categories of variations are recognized today: (1) the variations which have arisen abruptly and are qualitatively unlike the parent condition, and which breed true to type,—"sports" of Darwin, "mutations" of DeVries, "discontinuous variations" of Bateson; (2) the "fluctuating" variations which have arisen gradually and are merely quantitative variations of the parent condition—"Darwinian variations," "fluctuations" of DeVries, "continuous variations" of Bateson. It is well known that the selectionists claim that the latter type of variation is heritable[1] while on the other hand the mutationists and the pure line school either doubt or deny that fluctuating variations are inherited.

[1] Castle (1917); Jennings (1917).

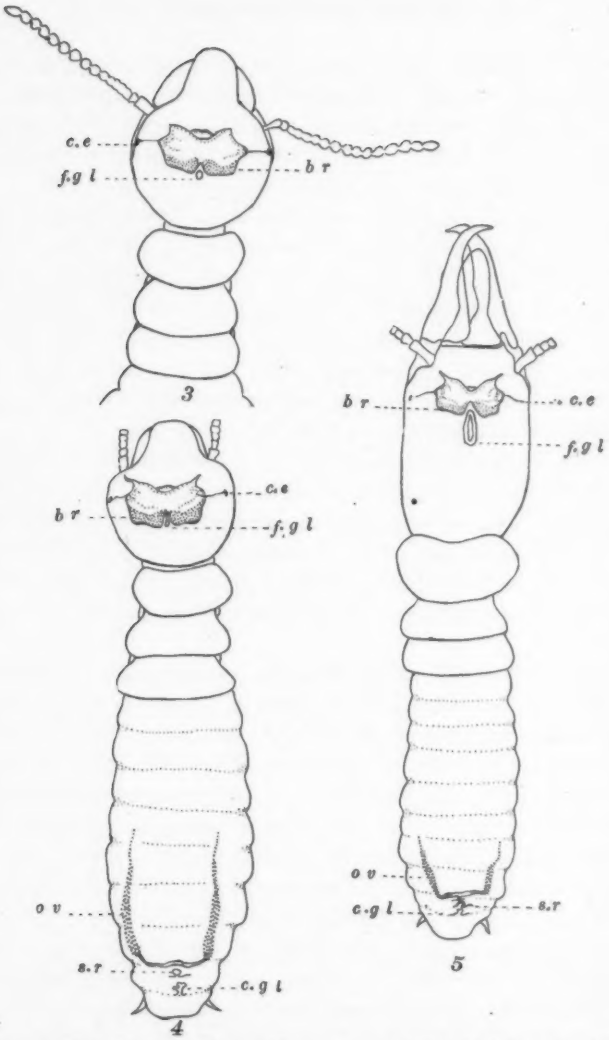

FIG. 3. *Reticulitermes flavipes*. Mature queen of the third form, head and thorax, drawn from a whole mount.

FIG. 4. *Reticulitermes flavipes*. Female worker, adult, drawn from a whole mount.

FIG. 5. *Reticulitermes flavipes*. Female soldier, adult, drawn from a whole mount. It should be noted that the parts of the reproductive system are separate and in an embryonic condition in the sterile workers and soldiers, Knower (1901).

Br, brain; *c.e*, compound eye; *c.gl*, colleterial gland; *f.gl*, frontal gland; *s.r*, seminal receptacle; *ov*, ovaries. Oc. 6, obj. 32 mm., drawn at stage level. Reduced one third. Drawn by C. B. T.

The question that presents itself to our minds is, therefore, in which of these two categories of variations do the termite castes belong? or, in other words, are the castes of termites, both in origin and at present, to be considered as fluctuating variations or as mutations?

Several lines of approach lead toward the solution of this problem: (1) the study of fossil insects, from the Cretaceous period when termites are said to appear[1] up to the present time; (2) the comparative morphology of the order of termites; (3) exact field observations upon termite biology; (4) breeding experiments to determine the type of progeny and the results of hybridization.

None of these lines of work have been thoroughly or exhaustively investigated, and the gaps in our knowledge are very wide. There are, however, a few scattered observations, to be summarized in this paper, drawn partly from the literature of the social insects, and partly from the field and laboratory studies of the two authors, which may be of value in the attempt to solve the problem of the variations known as termite castes.

In a recent paper, Wheeler (1917) has stated his opinion that the castes of ants represent continuous, *i. e.*, fluctuating variations. This view is based upon the study of the fossil ants of the Baltic amber, and upon comparative morphological data. "In most species of ants the constant and striking structural differences between the different castes would, at first sight, suggest that such forms as the apterous females, apterous males, soldiers and workers, must have arisen as so many saltatory variations, or mutants and that they survived and secured representation in the germ-plasm, because they happened to fulfill specialized and useful functions in the life of the colony. I believe, however, that this view of the castes, at least so far as their origin is concerned, cannot be maintained, because all the available evidence points to their being merely the surviving extremes of graduated and continuous series of forms, the annectant members of which have suffered phylogenetic suppression or extinction."

Handlirsch (1908), p. 1191, states that the number of termite genera was far greater in the Tertiary period than at present.

[1] Handlirsch (1908).

The field of termite morphology needs much revision, but a few significant facts may be stated here.

TABLE I.

THE CASTES OF *Reticulitermes flavipes* KOL.

Reproductive castes.	1. Adults of the first form.....	Long wings, or stubs after deälation; brain, compound eyes, and frontal gland large; mature sex organs largest of any caste; abundant pigment in skin. Aërial habit of swarming.
	2. Adults of the second form...	Short wing pads; brain, compound eyes, and frontal gland smaller; mature sex organs slightly smaller; less pigment. Subterranean mode of life.
	3. Adults of the third form.....	Entirely wingless; brain small, compound eyes, and frontal gland vestigial; mature sex organs much smaller; still less pigment. Subterranean mode of life.
Sterile castes.....	4. Workers.....	Wingless; brain small, compound eyes and frontal gland vestigial; sex organs embryonic; no pigment in body. Subterranean mode of life. Head broader than in the first and second reproductive forms, stouter mandibular muscles.
	5. Soldiers.....	Wingless; brain very small, compound eyes vestigial, frontal gland large; sex organs embryonic, even smaller than in the worker; no pigment in body. Subterranean mode of life. Elongate head, long mandibles, massive mandibular muscles.

An examination of the five castes of *R. flavipes* (Figs. 1–5, 6–8, 13–15, and Table I) shows that a gradation of characters may be traced throughout the series. In the three reproductive castes (Figs. 1, 2, 3), beginning with the winged adult of the first form, or its nymph of the first form which is practically similar in structure, an increasing loss of size is to be noted in the wings, in the brain, compound eyes, and frontal gland, and in the sex organs. The darkly pigmented body and the aërial habit in swarming are found only in the adults of the first form, less pigment and a wholly subterranean mode of life in the second and third forms. In the case of the worker (Fig. 4), and to a greater extent in the soldier (Fig. 5), there is a gain as well as a loss of characters, the gains being manifested in the larger head, pigmented in the soldier, and the larger mandibles and mandibular muscles; the losses, in the body pigment, the wings, brain, compound eyes, and sex organs. The frontal gland is merely vestigial in the worker, but is highly developed in the soldier. These castes might be interpreted either as gradations in a series of continuous or fluctuating variations, or as a series of regressive mutations, *i. e.*, mutations formed by the loss of characters, comparable to the series of mutations found in *Drosophila*, Morgan et al. (1915), Morgan and Bridges (1916). Should the former prove to be the case, then transitional or intermediate forms between the existing castes should be expected, but it must be remembered that mutations also may be arranged to form a structural series, even though they may not have originated in this order.

Up to the present time rather few intermediate forms have been described. The reason for this may be either that they have been overlooked thus far, or that they do not exist. The intermediate forms known at present are as follows:

In colonies of *Termopsis angusticollis* Walker, Heath (1903) describes fertile soldiers with wing buds, which produced "normal progeny." The question might well be asked what would be "normal progeny" under these circumstances?

Soldiers with vestiges of wing pads have been noted by the two authors in several species of *Calotermes: C. occidentis* Walker, and two new species from southern Florida; and by N. Banks in

TABLE II.

Genus.	Reproductive Castes.			Sterile Castes.			Variations.
	With Long Wings.	With Short Wing Pads.	Wingless.	Worker.	Soldier.	Nasutus.	
Termopsis Heer.....	1st form.	Forms with shorter wing pads than the normal 2d form.	3d form.		Soldier.		
Calotermes Hag.....	1st form.	2d form.	3d form.		Soldier.		*Calotermes* n. sp. from Fla., soldiers with abnormally shaped heads.
Neotermes Holm.....	1st form.	[2]	[2]		Soldier.		
Cryptotermes Bnks	1st form.	[2]	[2]		Soldier.		
Reticulitermes Holm.....	1st form.	2d form. (Also forms with both shorter and longer wing pads than the normal 2d form.)	3d form.	Worker.	Soldier.		*Reticulitermes* n.sp. from Pacific Coast, soldiers with longer heads.
Arrhinotermes Wasm	1st form.	2d form nymphs have been found, but no adults.	3d form.	Worker.	Soldier.		
Hamitermes Silv.....	1st form.	2d form.	[2]	Worker.	Soldier.		
Anoplotermes F. Müll. ..	1st form.	2d form.	[2]	Worker.			
Eutermes F. Müll.....	1st form.	2d form (in tropical species).	3d form (in tropical species).	Worker.		Nasutus.	

[2] This caste may exist in nature but it has not yet been found.

C. minor Hagen, and other species of *Calotermes*. Vestigial wing pads are evidently of frequent occurrence in this genus although these soldiers are in general not fertile.

The Rev. F. L. Odenbach, S.J., of Cleveland, Ohio, has kindly loaned his manuscript notes from which we quote. In one of Odenbach's colonies of *Reticulitermes flavipes*, an enlarged egg-laying queen, figured in manuscript notes and referred to by Snyder (1915, p. 56), has the abdomen distended and the abdominal tergites separated, but possesses long, well developed wing pads like a nymph of the first form.

Grassi (1893) has figured a queen which, in respect to the length of the wing pads, is an intermediate between the first and second forms in the species *R. lucifugus* Rossi.

Another peculiar form has been found in a colony of *Reticulitermes* n.sp. from Montana. This specimen (a male) has the head heavily chitinized and yellow in color as in the soldier; the mandibles and labrum are like those of the worker; but the head is slightly more elongate than the typical worker head. The total length of the specimen is nearer to that of the soldier. Unfortunately the antennæ are broken, so that the question whether it is a worker or soldier can not be determined from the number of segments. After staining, the frontal gland, the compound eyes, the brain, and the sex organs were identified as those of the worker caste (see Table I.). This specimen is evidently only a worker of abnormal development, and not, as was first thought, an intermediate form between the worker and the soldier.

An argument against the view that the termite castes are mutations is the fact that the five castes are constant in their structural characters and more or less constant in their occurrence throughout the very different and widely distributed genera of the three families of termites. In the native American genera listed in Table II. the first form or reproductive adult with long wings is found in every genus. Either a second or a third form adult is probably of similar occurrence. Sometimes both are present, sometimes one or the other is said to be lacking. This may be a real absence of the caste in question, or it may be due to incomplete field data. There is no worker caste in the primitive

genera *Termopsis* Heer, *Calotermes* Hag., *Neotermes* Holmg., and *Cryptotermes* Banks, but the activities of workers are carried on by the nymphs of the reproductive forms.

There is no soldier caste in the genus *Anoplotermes* Fritz Müller; this caste is also lacking in the genus *Eutermes* Fritz Müller, but here a new sterile caste has appeared: the "nasutus," a soldier-like form with a prolonged frontal process or "beak," which is often erroneously termed a soldier.

Many tropical species of termites have two types of soldiers and workers occurring within the species. The differences, however, are not marked structural ones but are quantitative, consisting mainly in the relative sizes of the two types, there being both "large" and "small" soldiers and "large" and "small" workers. These two types of worker or soldier or both, are said to occur in the genera *Rhinotermes* Hag., *Acanthotermes* Sjöst., *Termes* Linn., *Hamitermes* Silv., *Cornitermes* Wasm., *Eutermes* Fritz Müller, and *Leucotermes* Silv. In tropical species of *Eutermes* two types of nasuti, large and small, have been found. In our Nearctic termites, only two species have been seen with such differences in the soldiers. In two new species of *Calotermes* from Florida there are slight differences in the shape of the heads of soldiers *from the same colony*, and in *Reticulitermes* n. sp. from the Pacific coast there are also differences in the length of the heads of soldiers, but *from different colonies* (see Table II.).

FIELD OBSERVATIONS AND ARTIFICIAL BREEDING EXPERIMENTS.

With the collapse of the fantastic theories of Grassi and others in regard to the voluntary manufacture by the workers of "substitute" and "complemental" royal forms to replace the loss of the "true," winged, or first forms, we find ourselves at a loss to furnish the answer to the question how the different reproductive castes actually reproduce their kind today. Do the winged adults of the first form today give rise to the adults of the second and third forms as well as to the workers and soldiers? or does each fertile caste reproduce only its own fertile type together with the sterile forms?

Field observations upon species of the genus *Reticulitermes* by one author (T. E. S.) indicate that reproductive individuals

of the first form probably produce the nymphs of all three reproductive castes. At the proper season of the year—before the time of swarming—nymphs of the first and second forms are commonly found together in colonies, although colonies have been found in which no nymphs of the second form have been observed. Nymphs of the third form have never yet been found, which may be due to their resemblance to the young of the other castes; there is a possibility, however, that the individuals of the third form may be fertile workers, a point to be determined by morphological data, and now being investigated by one of the authors.

Both field observations and breeding experiments seem to indicate that the second and possibly the third forms produce, in addition to the sterile workers and soldiers, only their own fertile types, and never nymphs of the first form. In other words, the second and third form reproductive adults apparently breed true to their fertile types. In some artificial colonies with parent reproductive individuals of the second form, no reproductive forms but only sterile workers and soldiers have been produced. This seems to be the case in the two following experiments.

The Rev. F. L. Odenbach received in September, 1900, a small colony of termites, *Neotermes castaneus* Burm., from Florida. He placed these insects in an artificial nest and has continued to make observations on their habits. On July 1, 1902, eggs were found in the nest. In February, 1908, about one hundred and fifty members were present in the colony. In September, 1908, and again in June, 1909, reproductive forms were observed in the nest, much larger than the other members of the colony, and some with an enlarged abdomen, the body segments appearing as prominent chitinous bands, due to distention, a characteristic of the older termite queens. From Odenbach's description these were evidently reproductive individuals of the second or third form. In December, 1910, approximately two hundred individuals were in the nest. This colony was still alive in September, 1917. Workers and soldiers, but no forms with wing pads nor any winged adults, have been produced in this colony after seventeen years of breeding.

On August 2, 1915, one of the authors (T. E. S.) received a colony of a termite (*Reticulitermes* n. sp.) from Ivywild, Colo., found in a scrub white oak, and consisting of workers, soldiers and nymphs. On November 22, 1915, three females with abdomens considerably distended and two males with slightly distended abdomens were observed in the nest. These were reproductive individuals of the second form and had grayish and yellow pigmentation in the chitinized parts. While numerous eggs have been found every month in the year in this artificial colony, maintained indoors, and while the number of workers and soldiers has increased, no forms with wing pads or wings have been produced up to December, 1918, after three years of breeding, and the colony is large—several hundred members—and healthy. The criticism can scarcely be made that there has not been sufficient time for the production of winged forms, for even in recently established incipient colonies in nature the nymphs of the winged reproductive forms are produced after eighteen months.

Heath (1903) found winged adult termites of *Termopsis angusticollis* Walker swarming from nests in which males and females of the first form were present, the nests being only two years old and containing two hundred individuals.

One of the authors (T. E. S.) has made observations on the habits of termites since 1912, mainly in the southeastern United States, but in the season of 1917 during an extensive field trip through Florida, the southwest, the Rocky Mountains and the Pacific Coast regions. It may be stated with certainty that: (1) in long-established colonies, in which large fertilized queens of the second from occurred, no nymphs or winged adults of the first form have been found; (2) in all colonies in which queens of the third form were found, no nymphs or winged adults of the first form occurred; most of these colonies, however, were all small or young, that is, they had been recently established. In one large colony of *R. flavipes* in Virginia, with seventeen third form queens present, a few nymphs of the second form were found. Very few males of the third form have yet been found in the genus *Reticulitermes*, which is possibly due to their resemblance to young nymphs of the reproductive forms or to workers, and

to the fact that they are not so enlarged as the females. In the related genus *Arrhinotermes* males of the third form are of common occurrence.

The colonies of *Reticulitermes flavipes* and *virginicus* found in the southeastern United States in the spring, often contain nymphs of the second form, sometimes in large numbers, associated with either nymphs of the first form or winged sexual adults. These young reproductive types of the second form (Fig. 2) have attained their mature pigmentation at about the same time that the colonizing winged adults or reproductive types of the first form swarm, but after the swarm they are not found in the parent colonies. We may ask why they are produced and what becomes of them? They are not needed in the parent colony any more than the winged colonizing forms and it may be that they are impelled to leave the old colony by the same irresistible force that induces the swarm or flight. It may be, that, accompanied by workers and soldiers, they leave the parent colony by means of subterranean passages and establish a new colony. Since the origin of the castes is due to intrinsic causes, Thompson (1917), a certain proportionate number of these nymphs of the second form may be produced each year in long established colonies with parent first form adults. They would evidently be superfluous if the original reproductive forms of the parent colony were present, and might therefore be forced to migrate.

In certain colonies of termites, reproductive individuals of the second form occur with a small proportion of males to a large proportion of females. In the genus *Reticulitermes* as many as eight males together with thirty-two females, and fifteen males with twenty-eight females, both sexes of the second form, have been found (T. E. S.). In other colonies of *Reticulitermes* there are only reproductive individuals of the third form. Sometimes, again, a male of the first form is found with numerous (sixteen) mature females of the second form. Grassi would have described these second form females as "substitute" queens, produced by the workers to fill the place of a missing "true" queen.

It is an open question how they are to be accounted for with our present knowledge, and we have no exact data as to the

progeny. In one instance three queens of the third form and one queen of the second form were found together in the same colony, but both sexes of two different mature reproductive castes have never been found in one and the same colony of *Reticulitermes*. No experiments in crossing the different forms have yet been made.

The two authors hope to undertake a thorough investigation of the termite castes, especially of the three reproductive types, with an analysis of their breeding. This will be carried on by means of field and laboratory observations and by breeding experiments which will require several years to complete.

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DESCRIPTION OF PLATES.

FIGS. 6–9 are photographs of sections of the abdomens of termite queens. Figs. 10–12 are photomicrographs of the same sections made by John H. Paine, of the Bureau of Entomology. Figs. 13–15 are photographs of the living insects.

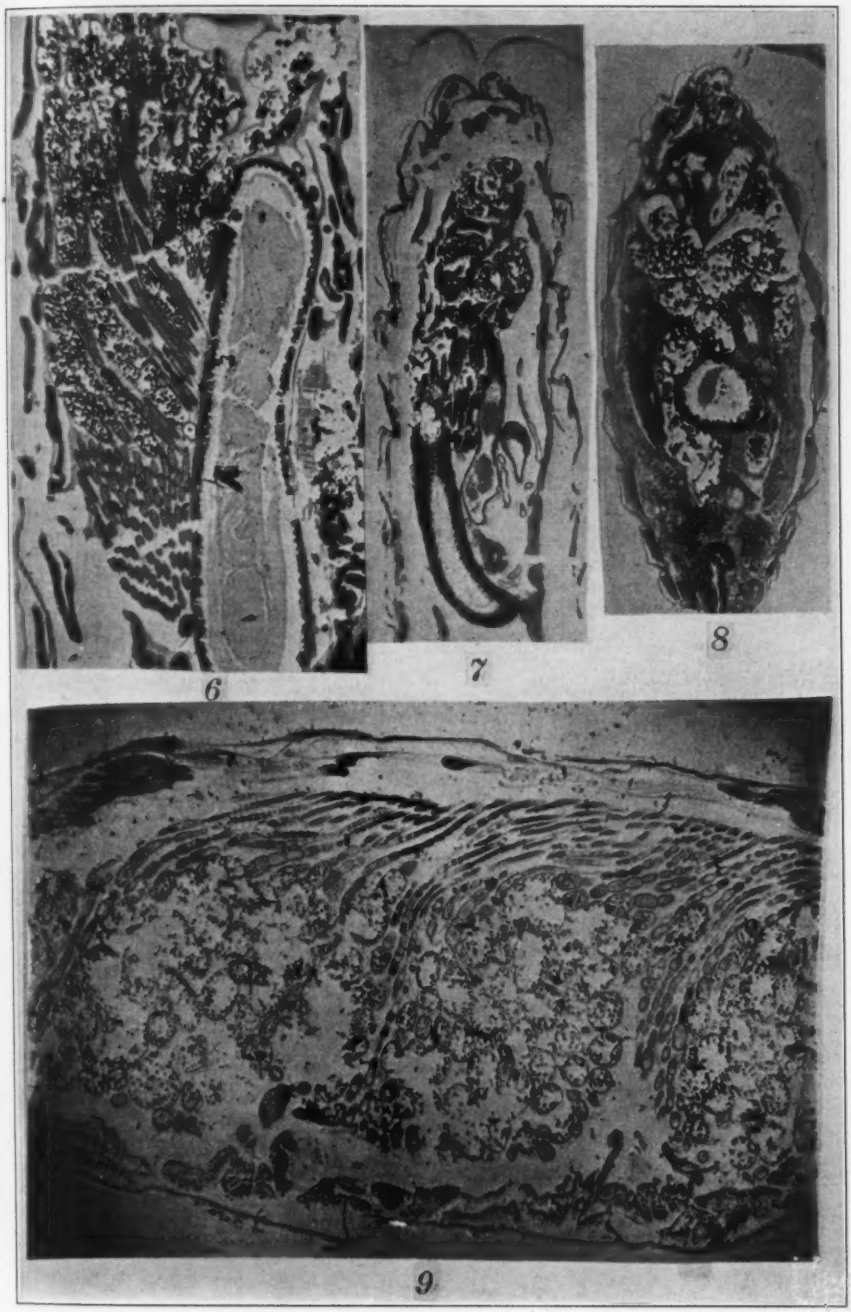
EXPLANATION OF PLATE 1.

FIG. 6. *Reticulitermes flavipes*. Photograph of a section of part of the abdomen of a mature first form queen, showing that the relative extent of the ovarian tubes is greater than in either of the other reproductive castes. × 20.

FIG. 7. *Reticulitermes flavipes*. Photograph of a section of the abdomen of mature queen of the second form. A portion of the oviduct is seen with the egg tubes opening into it laterally. × 20.

FIG. 8. *Reticulitermes flavipes*. Photograph of a portion of a section of the abdomen of a mature queen. The extent of the egg tubes is less than in the other two forms, but the ova shown happen to be of the oldest stage and are therefore larger. × 20.

FIG. 9. *Eutermes morio*. Photograph of a section of the abdomen of a mature queen of the first form, showing the enormous extent of the egg tubes and the varying sizes of the ova. × 20.



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EXPLANATION OF PLATE II.

FIG. 10. *Reticulitermes flavipes*. Mature queen of the first form, ovarian tubes. Photomicrographs of a portion of the same section shown in Fig. 6. × 65.

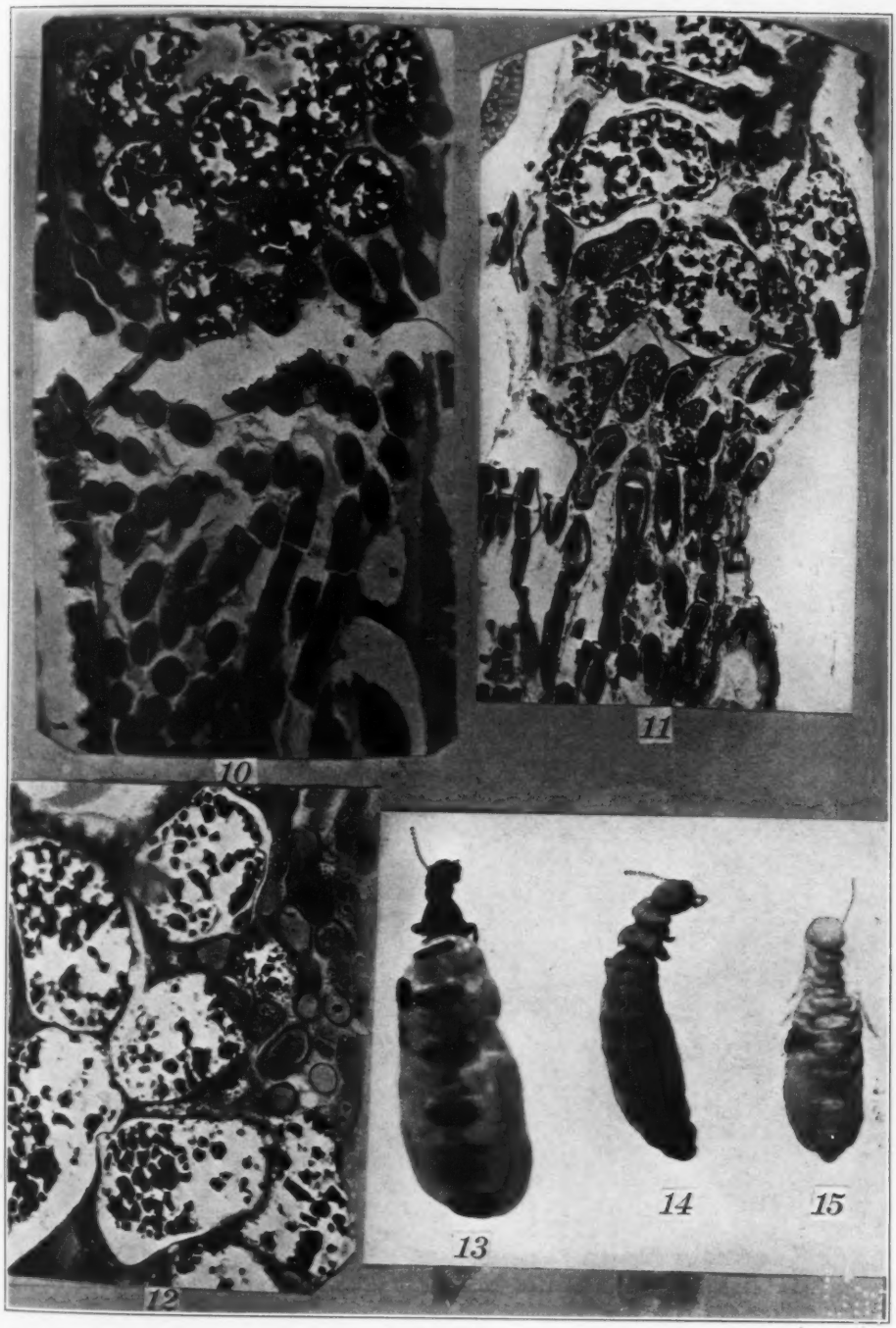
FIG. 11. *Reticulitermes flavipes*. Mature queen of the second form, ovarian tubes. Photomicrograph of a portion of the same section shown in Fig. 7. The oviduct and four entering egg tubes may be seen. × 65.

FIG. 12. *Reticulitermes flavipes*. Mature queen of the third form, ova. Photomicrograph of a portion of the same section seen in Fig. 8. × 65.

FIG. 13. *Reticulitermes flavipes*. Photograph of a mature queen of the first form. × 5.

FIG. 14. *Reticulitermes flavipes*. Photograph of a mature queen of the second form. × 5.

FIG. 15. *Reticulitermes flavipes*. Photograph of a mature queen of the third form. × 5.



C. B. THOMPSON AND T. E. SNYDER.

DEMONSTRATION OF THE AXIAL GRADIENTS BY MEANS OF POTASSIUM PERMANGANATE.

C. M. CHILD.

The existence of graded differences in susceptibility which are essentially quantitative rather than specific, and which show a definite relation to the physiological axes of organisms and parts has already been demonstrated for a large number of plant and animal forms by the various modifications of the susceptibility method, with a wide range of agents and conditions.[1] Additional data on other forms and with other methods, which support and further develop the original conception are as yet unpublished.

The two chief modifications of the susceptibility method, the so-called direct and acclimation methods, are concerned with the effects of different ranges of concentration or intensity of chemical and physical agents. The evidence obtained by these methods indicates that in general the regions or individuals in which the rate of oxidation or metabolism is highest are most susceptible to concentrations or intensities which either kill within a short time or are at least above the limit of acclimation or tolerance of the organism. The results of the acclimation method indicate that the regions or individuals with the highest rate of oxidation or metabolism acclimate or acquire tolerance most rapidly or most completely to concentrations or intensities which are within the limit of acclimation or tolerance. In short, the susceptibility method indicates, first, that both direct susceptibility to concentrations and intensities above the limit of tolerance and ability to acquire tolerance to lower concentrations and intensities of the same agents are associated with differences in the rate of the metabolic reactions of living protoplasm, and second, that the physiological axis *in its simplest form* is essentially a gradient in the rate of fundamental metabolic reactions. It must of course be borne in mind that the chemical reactions

[1] Child: '13, '14, '15*a*, *b*, '16*a*, *b*, *c*, *d*, '17*a*, *b*, *c*, *d*. Hyman, '16, '17.

themselves constitute only one aspect of such a gradient: associated with them are unquestionable differences in dispersion of colloids, permeability of membranes, enzyme content or activity, electrolytic dissociation and ion content, electrical potential, etc., and the point of view determines, at least to a considerable degree, which factor or group of factors in the complex we regard as fundamental. The essential fact is that these gradients in protoplasmic condition and activity exist and that they are not only characteristic features of physiological axes, but represent the earliest and simplest condition of such axes that we have thus far been able to discover.

The results given by the susceptibility method have been extended and confirmed by various other experimental methods and are not only in complete agreement with, but afford a satisfactory interpretation of a wide range of facts of observation and experiment, such, for example, as axial gradients in protoplasmic structure, in accumulation of yolk and other reserves, in rate of growth and ability to grow at the expense of other parts, in irritability and various other physiological activities.

During the past summer I have made extensive use of potassium permanganate as an agent for demonstrating the physiological gradients in protoplasm and making them directly visible as color gradients. Most of this work was done at the Puget Sound Biological Station at Friday Harbor, Wash., and I am indebted to the director, Dr. T. C. Frye and to the University of Washington for the privileges afforded me.

The Method.

In consequence of its powerful oxidizing action and the stain resulting from its reduction, potassium permanganate has long been used to some extent in histology, and certain investigators have attempted to determine with this and various other reagents the loci of reduction and of oxidation or of low and high oxygen content in cells and tissues. (See for example Golodetz and Unna, '09, P. G. Unna, '11, '13, '15, and various other papers.) The methods and results of these authors have been criticized (Oelze, '14*a*, *b*, *c*, Schmidt, '12, Schneider, '14) and their validity defended (Golodetz, '14, Unna, '15), but this controversy does

not affect the well-known fact that $K_2Mn_2O_8$ is readily reduced by protoplasm to MnO_2 which produces a brown coloration of the parts affected. The use of the permanganate for the purpose of demonstrating the gradients in protoplasm is not dependent in any way on Unna's methods and results and does not assume their validity, but is based on the simple assumption that the rate of reduction of permanganate and the consequent coloration of the protoplasm may be expected to show some relation to the physiological condition of the parts concerned, as regards their metabolism, oxidative ability, reducing capacity or avidity or demand for oxygen, in short, to some of the essential factors concerned in the protoplasmic gradients.

In most previous work with permanganate relatively high concentrations have been used, *e. g.*, 1 per cent. by Unna. In such concentrations it is of course a powerful oxidizing agent and extremely toxic, killing many of the lower animals almost instantaneously. In my own work much lower concentrations have been used ranging from *m*/1,000 down to *m*/100,000, *i. e.*, 0.0316 per cent. to 0.000316 per cent.[1] Even in a concentration of *m*/1,000, however, death usually occurs, at least in some parts of the body, within a few minutes. Most of my observations have been made with concentrations of *m*/10,000 or lower, since it has been found that the regional differences in rate of staining appear more clearly in the lower concentration, where the reduction and death occur slowly and progressively, than in the higher, where they are very rapid. In fact, when the organism is killed at once or very rapidly by the permanganate the differences characteristic of the living animal do not appear at all, or are very slight, and when it is killed by some other agent before exposure to permanganate, the differences do not appear.

The results thus far obtained with permanganate have not only confirmed the results of the susceptibility methods, so far as the same forms have been used, but in many cases the great delicacy of the staining reaction has brought out very clearly differences indistinctly or uncertainly shown by the cruder susceptibility methods with lethal concentrations. In fact the

[1] In making up solutions the formula $K_2Mn_2O_8$ with molecular wt. 316 was used. All concentrations are only approximate since solutions were not standardized.

permanganate method makes directly visible gradients such as the gradient in the unfertilized sea-urchin egg, which was not very satisfactorily demonstrated by the direct susceptibility methods (Child, '16*a*), but the existence of of which was inferred from the differential inhibition of development resulting from exposure of the unfertilized egg to inhibiting agents (Child, '16*c*). In various cleavage stages, hydroid planulæ, and echinoderm blastulæ and gastrulæ the color gradient is very distinct and uniform, even within the limits of a single blastomere in the earlier stages. In short the method is one of great beauty and delicacy and so entirely simple that it can readily be used for classes or lecture demonstration.

Practically the only precaution to be observed is to provide for uniform distribution and supply of permanganate to all part of the surface of the organism. The permanganate of course gradually disappears from the solution and if the volume of solution is not very large as compared with the protoplasmic surfaces it must be renewed from time to time. Moreover, in the case of organisms lying undisturbed for any considerable time on the bottom of the dish, the surface next the glass always stains less rapidly or less deeply than other regions. These difficulties are readily avoided by slight agitation of the solution at short intervals. Ciliary movement soon ceases in all except very low concentrations of permanganate so that even with motile developmental stages and free swimming protozoa as well as with non-motile forms or stages, frequent agitation of the solution is desirable.

Besides being useful as a staining agent for demonstrating the gradients as color gradients, permanganate can also be used, in at least many cases like KNC and other agents to demonstrate differences in susceptibility as gradients in death and disintegration. Thus far it has been less used in this way than as a staining agent, but it has been found for example that in various ciliate infusoria and early developmental stages of certain forms very low concentrations such as $m/50{,}000$ bring about death and disintegration before staining occurs. In all such cases observed the disintegration gradient is similar to the staining gradient. In the higher concentrations there is apparently more or less

coagulant or fixing action and so far as my observations go at present, the higher the concentration the less frequent is disintegration.

Brief Statement of Results.

The use of the method thus far has fully justified the assumption on which it was based, viz., that some relation would be found to exist between the rate of reduction of permanganate by living protoplasm as indicated by the rate or intensity of the stain resulting from deposition of MnO_2 and the physiological condition of the protoplasm, particularly its oxidative activity or capacity. It has been found that the regions indicated by other experimental methods and by structural and functional characteristics of the organism as regions of high oxidation rate are most rapidly or most deeply stained in permanganate and also show the highest susceptibility as regards death and disingration of the protoplasm in the proper concentrations. Moreover, the delicacy of the method indicates clearly the existence of certain minor differences in protoplasmic and metabolic condition, which cruder methods show less clearly or not at all.

Thus far permanganate has been used as a staining agent to demonstrate the gradients in various forms as opportunity offered. These forms include algæ, protozoa, hydroid and medusa forms, eggs and early developmental stages of hydrozoa, a siphonophore, young actinians, polyclad larvae, eggs and various early developmental stages of sea urchins, and ascidian tadpoles. Some observations have also been made on elongated organs such as the tentacles and branchiæ of the polychete, *Amphitrite*. The results as regards the existence of the gradients agree in general very closely with those obtained in other ways, except as regards some minor points shown less clearly or not at all by other methods.

In the algæ examined each active physiological axis of the thallus begins to stain at the apex and the stain progresses basipetally, but in the older regions of the body irregularities appear as with other methods (Child, '16*b*, '16*d*, '17*a*). In monosiphonous branching forms such as *Callithamnium* with elongated cells, the progress of the stain along a single cell can often be observed and in such forms also the gradient in proto-

plasmic disintegration observed with other agents can be clearly seen.

In a study of the determination of polarity by light in the egg of the alga *Fucus vesiculosus*, permanganate and various other agents were used. As is well known, the first steps in *Fucus* development are the cell division with the membrane at right angles to the direction of incident light and the outgrowth of the rhizoid on the side of the egg away from the light. The color gradient with permanganate as well as the susceptibility gradients with KNC and various other agents showed that in the early stages the rhizoid outgrowth stains first and is most susceptible, but that after three to five days a region of rapid staining and high susceptibility begins to arise at the opposite end of the developing plant and this second region becomes and remains the region of highest rate of oxidation as indicated by these methods and forms the apical growing region of the thallus. These data are merely recorded here and will be considered more fully at another time.

Perhaps the most interesting observations among those made on plants concern a diatom of the *Navicula* group. In this form the individuals, bound together by a transparent jelly-like secretion, form an alga-like structure, a pseudothallus, often reaching a length of several centimeters, which shows a definitely directed growth, regular and orderly bifurcation, and in short possesses all the external morphological characteristics of a multiaxiate, branching alga thallus. The appearance of this pseudothallus suggests very clearly that growth and multiplication of the diatoms composing it occur chiefly in the terminal regions of the branches, in fact, it is difficult to account for the orderly axiate habitus on any other basis. In the light of these facts, it is interesting to record that this pseudothallus composed of diatoms shows in permanganate a definite basipetal staining gradient in each branch or axis, for at least several millimeters from the tip, although further basally irregularities appear. A similar gradient in susceptibility has also been observed. In this form then although protoplasmic continuity from diatom to diatom supposedly does not exist, there is a definite, orderly relation, a gradient in physiological condition along each axis, as in axiate

algæ. Some sort of physiological correlation undoubtedly exists, and whatever its nature, it apparently gives rise to the same sort of order and unity as in other axiate forms. It can scarcely be supposed that in this case the correlation is accomplished by chemical substances given off by the individual diatoms, and the only other possibility is a transmissive relation of some sort, probably electrical in character (see R. S. Lillie, '17).

Among the protozoa observations have been made on *Noctiluca*, *Paramecium*, *Stentor* and *Spirostomum*. In *Noctiluca* the first traces of color appear on the surface of the membrane in the mouth region and the color spreads gradually over the membrane. The entoplasm later contracts and disintegrates into droplets before staining, but some slight indications of a basipetal gradient appear in this disintegration. The thickened flagellum of *Noctiluca* shows a well marked basipetal gradient.

In *Stentor* the color gradient in the ectoplasm is essentially the same as the susceptibility gradient already described (Child, '14), *i. e.*, basipetal, but the permanganate indicates a slight degree of "dorso-ventral" difference in that the staining progresses basipetally more rapidly on the oral than on the opposite side of the body. This difference along different meridians of the body has not been observed in susceptibility agents.

Spirostomum shows an ectoplasmic basipetal gradient both in color and in disintegration in permanganate, a short gradient in the opposite direction appearing at the basal end in a varying percentage of individuals. Other agents give essentially similar results. The secondary gradient in this form is very probably associated with the reversibility in the direction of locomotion which this species shows to a very high degree and with stimulation or irritation by the reagent for it apparently occurs only in those individuals which contract strongly and repeatedly in the solution.

In *Paramecium* the first effect of permanganate is, even in very low concentrations, a contraction of the ectoplasm beginning, and most extensive at the anterior end. This results in approach to spherical form, and in the lower concentrations the body often bursts either in the "anal" region or near one of the vacuoles.

In the higher concentrations a slight basipetal color gradient appears, but here again the posterior end commonly also stains almost as rapidly as the anterior, suggesting the existence of a secondary region of high activity there. Earlier work with susceptibility agents indicated a slight basipetal gradient in the majority of individuals, but the results were not as uniform as in many other species (Child, '14). It seems at least possible that certain irregularities in disintegration observed in the earlier work depend in part on the existence of a second region of rather high susceptibility in the posterior part of the body.

Five genera of hydromedusæ, *Phialidium*, *Æquorea*, *Mitrocoma*, *Sarsia* and one undetermined genus, tested repeatedly in permanganate, have shown in all cases a more rapid staining of the ectoderm of the subumbrella, than of the exumbrella. The difference is apparently greater in young than in old animals. These results agree with those of McClendon ('18) on oxygen consumption in the scyphomedusa *Cassiopea xamachana*. He found that the oxygen consumption of the subumbrella, exclusive of the manubrium is more than four times that of the exumbrella in the resting animal, with a still greater difference when pulsations occur. Each medusa tentacle also shows a basipetal color gradient.

In the colonial hydroids, *Bougainvillea*, *Obelia*, *Gonothyria* and one undetermined campanularian genus each hydranth body and each tentacle shows a basipetal color gradient, and with low concentrations a colony gradient also appears, *i. e.*, in a long stem with primary, secondary and perhaps tertiary branches the hydranths and growing tips of the apical region of the whole colony stain more rapidly than those of the basal region and the same differences appear on each of the longer primary branches. This colony gradient is clearly visible to the naked eye when the whole stem with its hydranths is placed in water after staining.

A basipetal color gradient also appears in the stems of the colony, the growing tips always staining much more rapidly and deeply than more basal regions. As regards these stems, however there is of course the possibility that the permanganate may penetrate the thinner perisarc of apical growing regions more readily than the thick perisarc of more basal levels, though, as a matter

of fact, penetration of the perisarc apparently occurs almost at once, and the differences in rate and depth of staining at different levels appear to be very much greater than the differences in perisarcal penetration.

The developmental stages of the egg of the hydromedusa, *Phialidium* from the ovarian egg to the hydroid have been examined with permanganate. In the ovarian egg, isolated by teasing, the region of the egg next to the free surface of the gonad stains most rapidly, with a color gradient from this region to the basal egg-pole. This gradient persists during fertilization, cleavage and to the advanced planula stage, the region of most rapid staining being the apical end of the embryo. In the late, elongated planula, just before attachment, a second region of rapid staining arises at what was originally the basal end, the planula attaches itself by its apical end, and the first hydroid arises as a bud from this second region of rapid staining at the basal end. The fact of attachment of the planula by the apical end and the development of the first hydranth from the original basal end has long been known to embryologists, and the MnO_2 color gradients merely serve to give some indication of the physiological conditions concerned. Apparently the first hydranth is physiologically a bud, a process of agamic reproduction in the planula, resulting from physiological isolation at the basal end, a process similar to the development of a second hydranth at the tip of the stolon in *Tubularia* (Child, '15*b*, pp. 91–92) the development of segments in annelids (Hyman, '16 Child, '17*d*), and many other reproductive processes in other forms (Child, '15*b*, Chapter V.). These results on hydrozoa are in agreement with those obtained by other methods, except that as regards unfertilized eggs, cleavage stages and general colony, gradients, the permanganate results are more distinct and definite.

The color gradients have been determined in one species of siphonophore belonging to the family Monophyidæ, a form with a single, elongated nectocalyx, from one side of which the stem or cœnosome bearing the groups of zoöids arises. The nectocalyx, which is of course a modified medusa, is morphologically bilaterally symmetrical and shows in permanganate, not only a

more rapid staining of the subumbrella than of the exumbrella like other hydromedusæ, but also a beautiful and striking bilaterality in the staining gradient, the side opposite the origin of the colony stem staining most rapidly, the stem side least rapidly. This difference is also visible with susceptibility methods, but less distinctly. In the groups of zoöids on the stem each zoöid, both nutritive and medusoid, shows a basipetal color gradient and the medusoid zoöids also show a bilateral gradient similar to that of the nectocalyx. In fact, these medusoids along the stem may later become nectocalyces, for each group, consisting of one nutritive, one medusoid zoöid, a group of tentacles and a bract, may become free and develop into a new siphonephore colony. The bilaterality of the color gradient in this form is a very striking feature.

Young actinians including the genus *Peachia* which is parasitic on hydromedusæ during its earlier life-history, and several other undetermined forms showed a very distinct basipetal color gradient in tentacles and body, and with certain concentrations a similar disintegration gradient.

In various embryonic and larval stages of polyclads, a beautiful basipetal color gradient appeared uniformly.

Unfertilized eggs of the sea urchin, *Strongylocentrotus franciscanus* show uniformly a very definite color gradient, although in the absence of definite landmarks it is not absolutely certain that this gradient is basipetal. However, the egg after fertilization and during cleavage does show a distinct basipetal gradient, even in each of the first two blastomeres, and these can be little doubt that this gradient is identical with that of the unfertilized egg. This gradient is also present in blastula and gastrula and is identical with the susceptibility gradient (Child, '16*a*).

The elongated tentacles and the branched branchial apparatus of the annelid, *Amphitrite* both exhibit very marked basipetal color gradients and in tadpoles of the solitary ascidian, *Corella willmeriana* the growing tail stains most rapidly at the tip with a definite gradient to the base, and the three papillæ of attachment which in this form grow out into temporary stolons during metamorphosis, but are later resorbed, are also regions of rapid staining. On the body of the larva staining progresses from the papillae more rapidly on the dorsal than on the ventral side.

These miscellaneous data on widely separated groups include the chief results obtained thus far with permanganate. It should perhaps be stated that in every case, except certain hydroids, the siphonophore and actinians at least ten individuals, and in the case of eggs and embryos hundreds or thousands were used, and the experiments were repeated at different times. With some of the hydroids only five or six branching stems from different colonies were used with the same results in all cases. In the case of the siphonophore three colonies were used, and since the results fully confirmed those obtained by other methods, and in each colony the same result could be observed in the different groups of zoöids, larger numbers were considered unnecessary. Of the actinians three to six individuals of each form tested were used, the uniformity of result in all the species making large numbers unnecessary.

Discussion.

The data briefly recorded above are sufficient to show that axial gradients in rapidity of staining with MnO_2 produced by the reduction of permanganate in low concentrations are at least of very wide occurrence in axiate organisms and organs. Moreover, in every case where data have already been obtained by other methods these have been confirmed by the color gradient, and in some cases slight symmetry gradients which were not visible or were indistinct with other methods have been clearly seen.

For various forms, including protozoa, medusa planulæ, echinoderm eggs and embryos and ascidian tadpoles, it has been demonstrated that the color gradient appears only when the living animals are brought into permanganate. When they are first killed without disintegration by some other agent, *e. g.*, mercurie chloride, alcohol, etc., and then brought into permanganate, staining is uniform, except where structural differences are concerned, and the gradient does not appear. Like the susceptibility gradients, the color gradients are thus features of living axiate organisms. This is undoubtedly true for all forms, but its truth has not been proved for all.

Only one further point need be considered here, viz., the question whether the color gradients are merely a result of differences

in rate of penetration of permanganate, in consequence of differences in permeability of different regions of cell or body, or whether they are more directly related to metabolic conditions in the protoplasm. With respect to this question it may be noted first that the color gradient begins to appear as a superficial gradient, apparently resulting from the deposition of MnO_2 on the cell surface. This is particularly evident in single cells, *e. g.*, *Noctiluca*, *Stentor*, eggs and blastomeres, where in optical section it can be seen that no appreciable penetration has occurred when the gradient has already begun to appear. Of course it cannot be denied that in such cases the permanganate has already penetrated a membrane of molecular thickness, but it certainly has not penetrated the visible structural membrane of the cell. In the protozoa the gradient is wholly or almost wholly limited to the ectoplasmic layer, but in eggs it apparently exists to some degree throughout the cytoplasm but appears first superficially. The facts seem at least to indicate that differences in a purely physical permeability of the cell membrane will not account for the color gradient any more than for the susceptibility gradient or the gradients in differential inhibition and acceleration of development.

As regards the question of permeability, however, it is doubtful whether we can isolate it as a purely physical condition from the chemical activity going on in the living protoplasm. Recent investigation indicates more and more clearly that such isolation is impossible. Without going into the matter at length, it is of interest to note that Osterhout has conceived changes in permeability in terms of chemical reaction, that R. S. Lillie in his later papers has emphasized the importance of metabolic reactions in relation to permeability and that recently Crozier ('18*a*, *b*) has concluded that for acids and alkalies the essential factor in stimulation is not an increase in physical permeability by depolarization, but rather a chemical reaction between the agent and some constituent of the receptor cell. And finally, even if we accept a purely physical theory of permeability and assume that the axial gradients in susceptibility to various agents and rate of staining by permanganate represent primarily gradients in permeability, there can be no doubt that such relatively per-

manent, localized and graded differences in permeability in such definite relation to the physiological axes, must be more or less closely associated with differences in metabolic condition, and that in general and within certain physiological limits we may expect to find a higher rate of oxidation associated with a region of higher permeability. Even on this basis then, the axial gradients in susceptibility, color, etc., would serve as indicators of metabolic gradients. But that a purely physical permeability of chemically inert membranes is not the primary factor in this relation is becoming increasingly evident, not merely in the recent modifications in the conception of permeability, but in the facts of susceptibility themselves. For example, differences in permeability of different cells or cell regions along an axis to neutral red and certain other "vital" dyes are usually slight and often inappreciable, although in some cases a distinct permeability gradient can be seen. Nevertheless axial gradients in susceptibility to these agents are very distinct, even within the limits of single cells, whether a gradient in penetration is distinguishable or not. Certainly physical permeability of the cell membrane is not the primary factor in such susceptibility gradients. Again, in the processes of acclimation and recovery that region of an axis which is most susceptible to the agent in high concentration or intensity undergoes acclimation most rapidly or completely in low concentrations or intensities, or recovers most rapidly and completely after temporary exposure within certain limits. Obviously the axial gradients of acclimation and recovery are very directly dependent upon metabolic rate in protoplasm and purely physical differences in permeability are of minor importance.

In the light of all the facts, then, I believe we are justified in concluding as regards the color gradients resulting from the protoplasmic reduction of permanganate; first, that they are indications of fundamental quantitative differences in the physiological condition of protoplasm, second, that such condition is very intimately related to the rate of oxidation, third, that the quantitative graded differences thus indicated represent the physiological axis in its simplest form.

SUMMARY.

The axial gradients in many plants and animals can be very clearly and beautifully demonstrated as color gradients by the use of low concentrations of $K_2Mn_2O_8$, the color being due to the reduction of the permanganate to MnO_2 by protoplasm.

Axial gradients have been demonstrated by this method in all axiate organisms thus far examined including several algæ, a diatom pseudothallus, four species of protozoa, various hydroids, hydromedusae and their developmental stages, a siphonophore, actinians, developmental stages of polyclads and echinoderms, axiate appendages of polychetes, and ascidian tadpoles.

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